

## **Albumin Fusion Proteins**

### **CROSS REFERENCE TO RELATED APPLICATION**

**[0000]** This application is a continuation of International Application No. PCT/US02/40891, filed December 23, 2002, which claims benefit under 35 USC 119(e) of U.S. Provisional Application Nos. 60/341,811, filed December 21, 2001; 60/350,358, filed January 24, 2002; 60/351,360, filed January 28, 2002; 60/359,370, filed February 26, 2002; 60/360,000, filed February 28, 2002; 60/367,500, filed March 27, 2002; 60/370,227, filed April 8, 2002; 60/378,950, filed May 10, 2002; 60/382,617, filed May 24, 2002; 60/383,123, filed May 28, 2002; 60/385,708, filed June 5, 2002; 60/394,625, filed July 10, 2002; 60/398,008, filed July 24, 2002; 60/402,131, filed August 9, 2002; 60/402,708, filed August 13, 2002; 60/411,426, filed September 18, 2002; 60/411,355, filed September 18, 2002; 60/414,984, filed October 2, 2002; 60/417,611, filed October 11, 2002; 60/420,246, filed October 23, 2002; and 60/423,623, filed November 5, 2002. All of the above listed applications are incorporated by reference herein.

### **BACKGROUND OF THE INVENTION**

**[0001]** The invention relates generally to Therapeutic proteins (including, but not limited to, at least one polypeptide, antibody, peptide, or fragment and variant thereof) fused to albumin or fragments or variants of albumin. The invention encompasses polynucleotides encoding therapeutic albumin fusion proteins, therapeutic albumin fusion proteins, compositions, pharmaceutical compositions, formulations and kits. Host cells transformed with the polynucleotides encoding therapeutic albumin fusion proteins are also encompassed by the invention, as are methods of making the albumin fusion proteins of the invention using these polynucleotides, and/or host cells.

**[0002]** Human serum albumin (HSA, or HA), a protein of 585 amino acids in its mature form (as shown in Figure 1 (SEQ ID NO:1038)), is responsible for a significant proportion of the osmotic pressure of serum and also functions as a carrier of endogenous and exogenous ligands. At present, HA for clinical use is produced by extraction from human blood. The production of recombinant HA (rHA) in microorganisms has been disclosed in EP 330 451 and EP 361 991.

**[0003]** Therapeutic proteins in their native state or when recombinantly produced, such as interferons and growth hormones, are typically labile molecules exhibiting short shelf-lives, particularly when formulated in aqueous solutions. The instability in these molecules when formulated for administration dictates that many of the molecules must be lyophilized and refrigerated at all times during storage, thereby rendering the molecules difficult to transport and/or store. Storage problems are particularly acute when pharmaceutical formulations must be stored and dispensed outside of the hospital environment.

**[0004]** Few practical solutions to the storage problems of labile protein molecules have been proposed. Accordingly, there is a need for stabilized, long lasting formulations of proteinaceous therapeutic molecules that are easily dispensed, preferably with a simple formulation requiring minimal post-storage manipulation.

## SUMMARY OF THE INVENTION

[0005] The present invention encompasses albumin fusion proteins comprising a Therapeutic protein (e.g., a polypeptide, antibody, or peptide, or fragment or variant thereof) fused to albumin or a fragment (portion) or variant of albumin. The present invention also encompasses polynucleotides comprising, or alternatively consisting of, nucleic acid molecules encoding a Therapeutic protein (e.g., a polypeptide, antibody, or peptide, or fragment or variant thereof) fused to albumin or a fragment (portion) or variant of albumin. The present invention also encompasses polynucleotides, comprising, or alternatively consisting of, nucleic acid molecules encoding proteins comprising a Therapeutic protein (e.g., a polypeptide, antibody, or peptide, or fragment or variant thereof) fused to albumin or a fragment (portion) or variant of albumin, that is sufficient to prolong the shelf life of the Therapeutic protein, and/or stabilize the Therapeutic protein and/or its activity in solution (or in a pharmaceutical composition) *in vitro* and/or *in vivo*. Albumin fusion proteins encoded by a polynucleotide of the invention are also encompassed by the invention, as are host cells transformed with polynucleotides of the invention, and methods of making the albumin fusion proteins of the invention and using these polynucleotides of the invention, and/or host cells.

[0006] In a preferred aspect of the invention, albumin fusion proteins include, but are not limited to, those encoded by the polynucleotides described in Table 2.

[0007] The invention also encompasses pharmaceutical formulations comprising an albumin fusion protein of the invention and a pharmaceutically acceptable diluent or carrier. Such formulations may be in a kit or container. Such kit or container may be packaged with instructions pertaining to the extended shelf life of the Therapeutic protein. Such formulations may be used in methods of treating, preventing, ameliorating or diagnosing a disease or disease symptom in a patient, preferably a mammal, most preferably a human, comprising the step of administering the pharmaceutical formulation to the patient.

[0008] In other embodiments, the present invention encompasses methods of preventing, treating, or ameliorating a disease or disorder. In preferred embodiments, the present invention encompasses a method of treating a disease or disorder listed in the "Preferred Indication: Y" column of Table 1 comprising administering to a patient in which such treatment, prevention or amelioration is desired an albumin fusion protein of the invention that comprises a Therapeutic protein or portion corresponding to a Therapeutic

protein (or fragment or variant thereof) disclosed in the "Therapeutic Protein: X" column of Table 1 (in the same row as the disease or disorder to be treated is listed in the "Preferred Indication: Y" column of Table 1) in an amount effective to treat, prevent or ameliorate the disease or disorder.

[0009] In one embodiment, an albumin fusion protein described in Table 1 or 2 has extended shelf life.

[0010] In a second embodiment, an albumin fusion protein described in Table 1 or 2 is more stable than the corresponding unfused Therapeutic molecule described in Table 1.

[0011] The present invention further includes transgenic organisms modified to contain the nucleic acid molecules of the invention (including, but not limited to, the polynucleotides described in Tables 1 and 2), preferably modified to express an albumin fusion protein of the invention.

#### **BRIEF DESCRIPTION OF THE FIGURES**

[0012] Figure 1A-D shows the amino acid sequence of the mature form of human albumin (SEQ ID NO:1038) and a polynucleotide encoding it (SEQ ID NO:1037).

[0013] Figure 2 shows the restriction map of the pPPC0005 cloning vector ATCC deposit PTA-3278.

[0014] Figure 3 shows the restriction map of the pSAC35 yeast *S. cerevisiae* expression vector (Sleep *et al.*, BioTechnology 8:42 (1990)).

[0015] Figure 4 shows the effect of various dilutions of EPO albumin fusion proteins encoded by DNA comprised in Construct ID NOS. (hereinafter CID) 1966 and 1981 and recombinant human EPO on the proliferation of TF-1 cells (see Examples 8 and 9). Cells were washed 3X to remove GM-CSF and plated at 10,000 cells/well for 72 hours in the presence of 3-fold dilutions of CID 1966 protein or CID 1981 protein. Concentrations used were calculated based on the weight of Epo alone, not HSA plus Epo. Recombinant human Epo (rhEpo) was used as the positive control and serially diluted 3 fold from 100 ng/ml to 0.01 ng/ml. Cells were exposed to 0.5 mCi/well of <sup>3</sup>H-thymidine for an additional 18 hours. (□) rhEpo; (▼) HSA-Epo 1981; (●) Epo-HSA 1966.

[0016] Figure 5 is a dose response analysis and shows the effect of various doses of recombinant human EPO and EPO albumin fusion proteins encoded by DNA comprised in CID 1966 and 1981 on the percent change in hematocrit from day 0 to day 7 (see Examples 8 and 9). 48 eight-week old female DBA/2NHsd mice were divided into 12 groups of 4



animals each. Recombinant human Epo (rhEpo) was administered subcutaneously at 0.5, 1.5, 4.5 and 12  $\mu\text{g/kg}$  on days 0, 2, 4, and 6. Epo albumin fusion proteins made from constructs CID 1966 and CID 1981 were administered subcutaneously at 2, 6, 18, and 54  $\mu\text{g/kg}$  on days 0, 2, 4, and 6. The higher doses of the Epo albumin fusion proteins allows a rough equimolar comparison with recombinant human Epo (note that the weight of the fusions is about 5.35 times the weight of non-glycosylated Epo). On days 0 and 7 of the experiment, the animals were bled via a tail vein and the hematocrit was determined by centrifugation. (■) rhEpo; (○) CID 1981; (▲) CID 1966.

[0017] Figure 6A shows the effect of various subcutaneous administrations of Epo albumin fusion proteins encoded by DNA comprised in CID 1966 and 1997, respectively, on the percent change in hematocrit from day 0 to day 8 (see Examples 8 and 10). \*,  $p < 0.005$  compared to rhEpo as determined by Mann-Whitney nonparametric analysis ( $n=6$ ).

[0018] Figure 6B shows the effect of subcutaneous administrations of Epo albumin fusion proteins encoded by DNA comprised in CID 1997 and 1966 on the percent change in hematocrit from day 0 to day 14 (see Examples 8 and 10). \*,  $p < 0.005$  compared to rhEpo as determined by Mann-Whitney nonparametric analysis ( $n=6$ ); \*\*,  $p < 0.05$  compared to rhEpo as determined by Mann-Whitney nonparametric analysis ( $n=6$ ).

[0019] Figure 7 shows the effect of various dilutions albumin fusion proteins encoded by DNA comprised in CID 1981 and 1997, respectively, on the proliferation of TF-1 cells (see Examples 9 and 10). Cells were washed 3X to remove GM-CSF and plated at 10,000 cells/well for 72 hours in the presence of 3-fold dilutions of Epo albumin fusion proteins encoded by CID 1981 or 1997. Equimolar amounts of rhEpo were used as a positive control (4.35 times less protein added since weight of non-glycosylated Epo is 20 kd, while Epo albumin fusion proteins are 87 kd). Cells were exposed to 0.5  $\mu\text{Ci/well}$  of  $^3\text{H}$ -thymidine for an additional 24 hours. (■) rhEpo Standard; (▲) CID 1981 (CHO); (○) CID 1997 (NSO).

[0020] Figure 8 shows the effect of various doses of recombinant human EPO (rhEpo) and EPO albumin fusion protein encoded by DNA comprised in construct 1997 (CID 1997) on the percent change in hematocrit from day 0 to day 8 (see Example 10). (▲) = rhEpo, (○) = CID 1997.

[0021] Figure 9 shows the effect of various dilutions of IL2 albumin fusion proteins encoded by DNA comprised in CID 1812 (see Example 15) on CTLL-2 proliferation.  $1 \times 10^4$  cells/well were seeded in a 96-well plate in a final volume of 200  $\mu\text{l}$  of complete medium

containing the indicated amount of IL2 albumin fusion protein (CID 1812). All samples were run in triplicate. The cells were incubated for 40 hours at 37°C, then 20 ul of Alamar Blue was added and cells incubated for 8 hours. Absorbance at 530/590 was used as a measure of proliferation.  $EC_{50} = 0.386 \pm 0.021$ . ( $\Delta$ ) = CID 1812.

**[0022]** Figure 10 shows the effect of IL2 albumin fusion protein encoded by DNA comprised in CID 1812 on RENCA tumor growth at day 21 (see Example 15). BALB/c mice (n=10) were injected SC (midflank) with  $10^5$  RENCA cells. 10 days later mice received 2 cycles (Day 10 to Day 14 and Days 17-21) of daily (QD) injections of rIL2 (0.9 mg/kg), IL2 albumin fusion protein (CID 1812 protein; 0.6 mg/kg), or PBS (Placebo) or injections every other day (QOD) of CID 1812 protein (0.6 mg/kg). The tumor volume was determined on Day 21 after RENCA inoculation. The data are presented in scatter analysis (each dot representing single animal). Mean value of each group is depicted by horizontal line. \*,  $p=0.0035$  between placebo control and CID 1812 protein. The number in parentheses indicates number of mice alive over the total number of mice per group. ( $\circ$ ) = Placebo; ( $\bullet$ ) = IL2; ( $\Delta$ ) = CID 1812 protein (QD); ( $\square$ ) = CID 1812 protein (QOD).

**[0023]** Figure 11 shows the effect of various dilutions of GCSF albumin fusion proteins encoded by DNA comprised in CID 1642 and 1643 on NFS-60 cell proliferation (see Examples 19 and 20). ( $\blacksquare$ ) = CID 1642; ( $\blacktriangle$ ) = CID 1643; ( $\circ$ ) = HSA.

**[0024]** Figure 12 shows the effect of recombinant human GCSF (Neupogen) and GCSF albumin fusion protein on total white blood cell count (see Example 19). Total WBC ( $10^3$  cells/ul) on each day are presented as the group mean  $\pm$  SEM. GCSF albumin fusion protein was administered sc at either 25 or 100 ug/kg every 4 days x 4 (Q4D), or at 100 ug/kg every 7 days x 2 (Q7D). Data from Days 8 and 9 for GCSF albumin fusion protein 100 ug/kg Q7 are presented as Days 9 and 10, respectively, to facilitate comparison with other groups. Controls were saline vehicle administered SC every 4 days x 4 (Vehicle Q4D), or Neupogen administered SC daily x 14 (Neupogen 5 ug/kg QD). The treatment period is considered Days 1-14, and the recovery period, Days 15-28.

**[0025]** Figure 13 shows the effect of various dilutions of IFNb albumin fusion proteins encoded by DNA comprised in CID 2011 and 2053 on SEAP activity in the ISRE-SEAP/293F reporter cells (see Example 25). Proteins were serially diluted from  $5e-7$  to  $1e-14$  g/ml in DMEM/10% FBS and used to treat ISRE-SEAP/293F reporter cells. After 24 hours supernatants were removed from reporter cells and assayed for SEAP activity. IFNb albumin fusion protein was purified from three stable clones: 293F/#2011, CHO/#2011 and

NSO/#2053. Mammalian derived IFN $\beta$ , Avonex, came from Biogen and was reported to have a specific activity of 2.0e5 IU/ $\mu$ g.

**[0026]** Figure 14 illustrates the steady-state levels of insulin mRNA in INS-1 (832/13) cells after treatment with GLP-1 or GLP-1 albumin fusion protein encoded by construct ID 3070 (CID 3070 protein). Both GLP-1 and the CID 3070 protein stimulate transcription of the insulin gene in INS-1 cells. The first bar (black) represents the untreated cells. Bars 2-4 (white) represent cells treated with the indicated concentrations of GLP-1. Bars 5-7 (gray) represent cells treated with the indicated concentrations of CID 3070 protein.

**[0027]** Figure 15 compares the anti-proliferative activity of IFN albumin fusion protein encoded by CID 3165 (CID 3165 protein) and recombinant IFN $\alpha$  (rIFN $\alpha$ ) on Hs294T melanoma cells. The cells were cultured with varying concentrations of either CID 3165 protein or rIFN $\alpha$  and proliferation was measured by BrdU incorporation after 3 days of culture. CID 3165 protein caused measurable inhibition of cell proliferation at concentrations above 10 ng/ml with 50% inhibition achieved at approximately 200 ng/ml. (■) = CID 3165 protein, (◆) = rIFN $\alpha$ .

**[0028]** Figure 16 shows the effect of various dilutions of IFN $\alpha$  albumin fusion proteins on SEAP activity in the ISRE-SEAP/293F reporter cells. One preparation of IFN $\alpha$  fused upstream of albumin (◆) was tested, as well as two different preparations of IFN $\alpha$  fused downstream of albumin (▲) and (■).

**[0029]** Figure 17 shows the effect of time and dose of IFN $\alpha$  albumin fusion protein encoded by DNA comprised in construct 2249 (CID 2249 protein) on the mRNA level of OAS (p41) in treated monkeys (see Example 31). Per time point: first bar = Vehicle control, 2<sup>nd</sup> bar = 30  $\mu$ g/kg CID 2249 protein day 1 iv, third bar = 30  $\mu$ g/kg CID 2249 protein day 1 sc, 4<sup>th</sup> bar = 300  $\mu$ g/kg CID 2249 protein day 1 sc, 5<sup>th</sup> bar = 40  $\mu$ g/kg recombinant IFN $\alpha$  day 1, 3 and 5 sc.

**[0030]** Figure 18 shows the effect of various dilutions of insulin albumin fusion proteins encoded by DNA comprised in constructs 2250 and 2276 on glucose uptake in 3T3-L1 adipocytes (see Examples 33 and 35).

**[0031]** Figure 19 shows the effect of various GCSF albumin fusion proteins, including those encoded by CID #1643 and #2702 (L-171, see Example 114), on NFS cell proliferation. The horizontal dashed line indicates the minimum level of detection.

## DETAILED DESCRIPTION

## **Definitions**

**[0032]** The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

**[0033]** As used herein, “polynucleotide” refers to a nucleic acid molecule having a nucleotide sequence encoding a fusion protein comprising, or alternatively consisting of, at least one molecule of albumin (or a fragment or variant thereof) joined in frame to at least one Therapeutic protein X (or fragment or variant thereof); a nucleic acid molecule having a nucleotide sequence encoding a fusion protein comprising, or alternatively consisting of, the amino acid sequence of SEQ ID NO:Y (as described in column 6 of Table 2) or a fragment or variant thereof; a nucleic acid molecule having a nucleotide sequence comprising or alternatively consisting of the sequence shown in SEQ ID NO:X; a nucleic acid molecule having a nucleotide sequence encoding a fusion protein comprising, or alternatively consisting of, the amino acid sequence of SEQ ID NO:Z; a nucleic acid molecule having a nucleotide sequence encoding an albumin fusion protein of the invention generated as described in Table 2 or in the Examples; a nucleic acid molecule having a nucleotide sequence encoding a Therapeutic albumin fusion protein of the invention, a nucleic acid molecule having a nucleotide sequence contained in an albumin fusion construct described in Table 2, or a nucleic acid molecule having a nucleotide sequence contained in an albumin fusion construct deposited with the ATCC (as described in Table 3).

**[0034]** As used herein, “albumin fusion construct” refers to a nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide encoding at least one molecule of albumin (or a fragment or variant thereof) joined in frame to at least one polynucleotide encoding at least one molecule of a Therapeutic protein (or fragment or variant thereof); a nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide encoding at least one molecule of albumin (or a fragment or variant thereof) joined in frame to at least one polynucleotide encoding at least one molecule of a Therapeutic protein (or fragment or variant thereof) generated as described in Table 2 or in the Examples; or a nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide encoding at least one molecule of albumin (or a fragment or variant thereof) joined in frame to at least one polynucleotide encoding at least one molecule of a Therapeutic protein (or fragment or variant thereof), further comprising, for example, one or more of the following elements: (1) a functional self-replicating vector (including but not limited to, a shuttle vector, an expression vector, an integration vector, and/or a replication system), (2) a region for

initiation of transcription (e.g., a promoter region, such as for example, a regulatable or inducible promoter, a constitutive promoter), (3) a region for termination of transcription, (4) a leader sequence, and (5) a selectable marker. The polynucleotide encoding the Therapeutic protein and albumin protein, once part of the albumin fusion construct, may each be referred to as a “portion,” “region” or “moiety” of the albumin fusion construct.

**[0035]** The present invention relates generally to polynucleotides encoding albumin fusion proteins; albumin fusion proteins; and methods of treating, preventing, or ameliorating diseases or disorders using albumin fusion proteins or polynucleotides encoding albumin fusion proteins. As used herein, “albumin fusion protein” refers to a protein formed by the fusion of at least one molecule of albumin (or a fragment or variant thereof) to at least one molecule of a Therapeutic protein (or fragment or variant thereof). An albumin fusion protein of the invention comprises at least a fragment or variant of a Therapeutic protein and at least a fragment or variant of human serum albumin, which are associated with one another by genetic fusion (i.e., the albumin fusion protein is generated by translation of a nucleic acid in which a polynucleotide encoding all or a portion of a Therapeutic protein is joined in-frame with a polynucleotide encoding all or a portion of albumin). The Therapeutic protein and albumin protein, once part of the albumin fusion protein, may each be referred to as a “portion”, “region” or “moiety” of the albumin fusion protein (e.g., a “Therapeutic protein portion” or an “albumin protein portion”). In a highly preferred embodiment, an albumin fusion protein of the invention comprises at least one molecule of a Therapeutic protein X or fragment or variant of thereof (including, but not limited to a mature form of the Therapeutic protein X) and at least one molecule of albumin or fragment or variant thereof (including but not limited to a mature form of albumin).

**[0036]** In a further preferred embodiment, an albumin fusion protein of the invention is processed by a host cell and secreted into the surrounding culture medium. Processing of the nascent albumin fusion protein that occurs in the secretory pathways of the host used for expression may include, but is not limited to signal peptide cleavage; formation of disulfide bonds; proper folding; addition and processing of carbohydrates (such as for example, N- and O- linked glycosylation); specific proteolytic cleavages; and assembly into multimeric proteins. An albumin fusion protein of the invention is preferably in the processed form. In a most preferred embodiment, the “processed form of an albumin fusion protein” refers to an albumin fusion protein product which has undergone N- terminal signal peptide cleavage, herein also referred to as a “mature albumin fusion protein”.

**[0037]** In several instances, a representative clone containing an albumin fusion construct of the invention was deposited with the American Type Culture Collection (herein referred to as "ATCC®"). Furthermore, it is possible to retrieve a given albumin fusion construct from the deposit by techniques known in the art and described elsewhere herein. The ATCC® is located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA. The ATCC® deposits were made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedure.

**[0038]** In one embodiment, the invention provides a polynucleotide encoding an albumin fusion protein comprising, or alternatively consisting of, a Therapeutic protein and a serum albumin protein. In a further embodiment, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a Therapeutic protein and a serum albumin protein. In a preferred embodiment, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a Therapeutic protein and a serum albumin protein encoded by a polynucleotide described in Table 2. In a further preferred embodiment, the invention provides a polynucleotide encoding an albumin fusion protein whose sequence is shown as SEQ ID NO:Y in Table 2. In other embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a biologically active and/or therapeutically active fragment of a Therapeutic protein and a serum albumin protein. In other embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a biologically active and/or therapeutically active variant of a Therapeutic protein and a serum albumin protein. In preferred embodiments, the serum albumin protein component of the albumin fusion protein is the mature portion of serum albumin. The invention further encompasses polynucleotides encoding these albumin fusion proteins.

**[0039]** In further embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a Therapeutic protein, and a biologically active and/or therapeutically active fragment of serum albumin. In further embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a Therapeutic protein and a biologically active and/or therapeutically active variant of serum albumin. In preferred embodiments, the Therapeutic protein portion of the albumin fusion protein is the mature portion of the Therapeutic protein. In a further preferred embodiment, the Therapeutic protein portion of the albumin fusion protein is the extracellular soluble domain of the Therapeutic protein. In an alternative embodiment, the Therapeutic protein

portion of the albumin fusion protein is the active form of the Therapeutic protein. The invention further encompasses polynucleotides encoding these albumin fusion proteins.

[0040] In further embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a biologically active and/or therapeutically active fragment or variant of a Therapeutic protein and a biologically active and/or therapeutically active fragment or variant of serum albumin. In preferred embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, the mature portion of a Therapeutic protein and the mature portion of serum albumin. The invention further encompasses polynucleotides encoding these albumin fusion proteins.

### **Therapeutic proteins**

[0041] As stated above, a polynucleotide of the invention encodes a protein comprising or alternatively consisting of, at least a fragment or variant of a Therapeutic protein and at least a fragment or variant of human serum albumin, which are associated with one another, preferably by genetic fusion.

[0042] An additional embodiment includes a polynucleotide encoding a protein comprising or alternatively consisting of at least a fragment or variant of a Therapeutic protein and at least a fragment or variant of human serum albumin, which are linked with one another by chemical conjugation.

[0043] As used herein, "Therapeutic protein" refers to proteins, polypeptides, antibodies, peptides or fragments or variants thereof, having one or more therapeutic and/or biological activities. Therapeutic proteins encompassed by the invention include but are not limited to, proteins, polypeptides, peptides, antibodies, and biologics. (The terms peptides, proteins, and polypeptides are used interchangeably herein.) It is specifically contemplated that the term "Therapeutic protein" encompasses antibodies and fragments and variants thereof. Thus a protein of the invention may contain at least a fragment or variant of a Therapeutic protein, and/or at least a fragment or variant of an antibody. Additionally, the term "Therapeutic protein" may refer to the endogenous or naturally occurring correlate of a Therapeutic protein.

[0044] By a polypeptide displaying a "therapeutic activity" or a protein that is "therapeutically active" is meant a polypeptide that possesses one or more known biological and/or therapeutic activities associated with a therapeutic protein such as one or more of the Therapeutic proteins described herein or otherwise known in the art. As a non-limiting

example, a "Therapeutic protein" is a protein that is useful to treat, prevent or ameliorate a disease, condition or disorder. As a non-limiting example, a "Therapeutic protein" may be one that binds specifically to a particular cell type (normal (e.g., lymphocytes) or abnormal e.g., (cancer cells)) and therefore may be used to target a compound (drug, or cytotoxic agent) to that cell type specifically.

[0045] For example, a non-exhaustive list of "Therapeutic protein" portions which may be comprised by an albumin fusion protein of the invention includes, but is not limited to, erythropoietin (EPO), IL-2, G-CSF, Insulin, Calcitonin, Growth Hormone, IFN-alpha, IFN-beta, PTH, TR6 (International Publication No. WO 98/30694), BLYS, BLYS single chain antibody, Resistin, Growth hormone releasing factor, VEGF-2, KGF-2, D-SLAM, KDI, and TR2, GLP-1, Extendin 4, and GM-CSF.

[0046] Interferon hybrids may also be fused to the amino or carboxy terminus of albumin to form an interferon hybrid albumin fusion protein. Interferon hybrid albumin fusion protein may have enhanced, or alternatively, suppressed interferon activity, such as antiviral responses, regulation of cell growth, and modulation of immune response (Lebleu et al., *PNAS USA*, 73:3107-3111 (1976); Gresser et al., *Nature*, 251:543-545 (1974); and Johnson, *Texas Reports Biol Med*, 35:357-369 (1977)). Each interferon hybrid albumin fusion protein can be used to treat, prevent, or ameliorate viral infections (e.g., hepatitis (e.g., HCV); or HIV), multiple sclerosis, or cancer.

[0047] In one embodiment, the interferon hybrid portion of the interferon hybrid albumin fusion protein comprises an interferon alpha-interferon alpha hybrid (herein referred to as an alpha-alpha hybrid). For example, the alpha-alpha hybrid portion of the interferon hybrid albumin fusion protein consists, or alternatively comprises, of interferon alpha A fused to interferon alpha D. In a further embodiment, the A/D hybrid is fused at the common BgIII restriction site to interferon alpha D, wherein the N-terminal portion of the A/D hybrid corresponds to amino acids 1-62 of interferon alpha A and the C-terminal portion corresponds to amino acids 64-166 of interferon alpha D. For example, this A/D hybrid would comprise the amino acid sequence:

CDLPQTHSLGSRRTLMLLAQMRX<sub>1</sub>ISLFSCLKDRHDFGFPQEFGNQFQKAETIPVLHE  
MIQQIFNLFTTKDSSAAWDEDLLDKFCTELYQQNLNLEACVMQEERVGETPLMNX<sub>2</sub>D  
SILAVKKYFRRITLYLTEKKYSPCAWEVVRAEIMRSLSLSTNLQERLRRKE (SEQ ID  
NO:1326), wherein the X<sub>1</sub> is R or K and the X<sub>2</sub> is A or V (see, for example, Construct ID  
#2875). In an additional embodiment, the A/D hybrid is fused at the common PvuIII



restriction site, wherein the N-terminal portion of the A/D hybrid corresponds to amino acids 1-91 of interferon alpha A and the C-terminal portion corresponds to amino acids 93-166 of interferon alpha D. For example, this A/D hybrid would comprise the amino acid sequence:

CDLPQTHSLGSRRTLMLLAQMRX<sub>1</sub>ISLFSCLKDRHDFGFPQEFGNQFQKAETIPVLHE  
MIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDLEACVMQEERVGETPLMNX<sub>2</sub>D  
SILAVKKYFRRITLYLTEKKYSPCAWEVVRAEIMRSLSLSTNLQERLRRKE (SEQ ID  
NO:1311), wherein the X<sub>1</sub> is R or K and the second X<sub>2</sub> is A or V (see, for example, Construct  
ID #2872). These hybrids are further described in U.S. Patent No. 4,414,510, which is hereby  
incorporated by reference in its entirety.

**[0048]** In an additional embodiment, the alpha-alpha hybrid portion of the interferon  
hybrid albumin fusion protein consists, or alternatively comprises, of interferon alpha A fused  
to interferon alpha F. In a further embodiment, the A/F hybrid is fused at the common PvuIII  
restriction site, wherein the N-terminal portion of the A/F hybrid corresponds to amino acids  
1-91 of interferon alpha A and the C-terminal portion corresponds to amino acids 93-166 of  
interferon alpha F. For example, this A/F hybrid would comprise the amino acid sequence:

CDLPQTHSLGSRRTLMLLAQMRX<sub>1</sub>ISLFSCLKDRHDFGFPQEFGNQFQKAETIPVLHE  
MIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDMEACVIQEVGVEETPLMNVDS  
ILAVKKYFQRITLYLTEKKYSPCAWEVVRAEIMRSFSLSKIFQERLRRKE (SEQ ID

NO:1321), wherein X is either R or K (see, for example, Construct ID #2874). These hybrids  
are further described in U.S. Patent No. 4,414,510, which is hereby incorporated by reference  
in its entirety. In a further embodiment, the alpha-alpha hybrid portion of the interferon  
hybrid albumin fusion protein consists, or alternatively comprises, of interferon alpha A fused  
to interferon alpha B. In an additional embodiment, the A/B hybrid is fused at the common  
PvuIII restriction site, wherein the N-terminal portion of the A/B hybrid corresponds to amino  
acids 1-91 of interferon alpha A and the C-terminal portion corresponds to amino acids 93-  
166 of interferon alpha B. For example, this A/B hybrid would comprise an amino acid  
sequence:

CDLPQTHSLGSRRTLMLLAQMRX<sub>1</sub>ISLFSCLKDRHDFGFPQEFGNQFQKAETIPVLHE  
MIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDLEX<sub>2</sub>X<sub>3</sub>X<sub>4</sub>X<sub>5</sub>QEVGVIESPLMYE  
DSILAVRKYFQRITLYLTEKKYSSCAWEVVRAEIMRSFSLINLQKRLKSKE (SEQ ID

NO:1316), wherein the X<sub>1</sub> is R or K and X<sub>2</sub> through X<sub>5</sub> is SCVM or VLCD (see, for example,  
Construct ID #2873). These hybrids are further described in U.S. Patent No. 4,414,510,  
which is hereby incorporated by reference in its entirety.

**[0049]** In another embodiment, the interferon hybrid portion of the interferon hybrid albumin fusion protein comprises an interferon beta-interferon alpha hybrid (herein referred to as a beta-alpha hybrid). For example, the beta-alpha hybrid portion of the interferon hybrid albumin fusion protein consists, or alternatively comprises, of interferon beta-1 fused to interferon alpha D (also referred to as interferon alpha-1). In a further embodiment, the beta-1/alpha D hybrid is fused wherein the N-terminal portion corresponds to amino acids 1-73 of interferon beta-1 and the C-terminal portion corresponds to amino acids 74-167 of interferon alpha D. For example, this beta-1/alpha D hybrid would comprise an amino acid sequence: MSYNLLGFLQRSSNFQCQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKEDAAL TIYEMLQNIFAIFRQDSSAAWDEDLLDKFCTELYQQLNDEACVMQEERVGETPLMN XDSILAVKKYFRRITLYLTEKKYSPCAWEVVRAEIMRSLSLSTNLQERLRKE (SEQ ID NO:2130), wherein X is A or V. These hybrids are further described in U.S. Patent No. 4,758,428, which is hereby incorporated by reference in its entirety.

**[0050]** In another embodiment, the interferon hybrid portion of the interferon hybrid albumin fusion protein comprises an interferon alpha-interferon beta hybrid (herein referred to as a alpha-beta hybrid). For example, the alpha-beta hybrid portion of the interferon hybrid albumin fusion protein consists, or alternatively comprises, of interferon alpha D (also referred to as interferon alpha-1) fused to interferon beta-1. In a further embodiment, the alpha D/beta-1 hybrid is fused wherein the N-terminal portion corresponds to amino acids 1-73 of interferon alpha D and the C-terminal portion corresponds to amino acids 74-166 of interferon beta-1. For example, this alpha D/beta-1 hybrid would have an amino acid sequence:

MCDLPETHSLDNRRTLMLLAQMSRISPSSCLMDRHDGFGFPQEEFDGNQFQKAPASV LHELIQQIFNLFTTKDSSSTGWNETIVENLLANVYHQINHLKTVLEEKLEKEDFTRGKL MSSLHLKRYYYGRILHYLKAKEYSHCAWTIVRVEILRNIFYFINRLTGYLRLN (SEQ ID NO:2131). These hybrids are further described in U.S. Patent No. 4,758,428, which is hereby incorporated by reference in its entirety.

**[0051]** In further embodiments, the interferon hybrid portion of the interferon hybrid albumin fusion proteins may comprise additional combinations of alpha-alpha interferon hybrids, alpha-beta interferon hybrids, and beta-alpha interferon hybrids. In additional embodiments, the interferon hybrid portion of the interferon hybrid albumin fusion protein may be modified to include mutations, substitutions, deletions, or additions to the amino acid sequence of the interferon hybrid. Such modifications to the interferon hybrid albumin fusion

proteins may be made, for example, to improve levels of production, increase stability, increase or decrease activity, or confer new biological properties.

**[0052]** The above-described interferon hybrid albumin fusion proteins are encompassed by the invention, as are host cells and vectors containing polynucleotides encoding the polypeptides. In one embodiment, a interferon hybrid albumin fusion protein encoded by a polynucleotide as described above has extended shelf life. In an additional embodiment, a interferon hybrid albumin fusion protein encoded by a polynucleotide described above has a longer serum half-life and/or more stabilized activity in solution (or in a pharmaceutical composition) *in vitro* and/or *in vivo* than the corresponding unfused interferon hybrid molecule.

**[0053]** In another non-limiting example, a "Therapeutic protein" is a protein that has a biological activity, and in particular, a biological activity that is useful for treating, preventing or ameliorating a disease. A non-inclusive list of biological activities that may be possessed by a Therapeutic protein includes, enhancing the immune response, promoting angiogenesis, inhibiting angiogenesis, regulating endocrine function, regulating hematopoietic functions, stimulating nerve growth, enhancing an immune response, inhibiting an immune response, or any one or more of the biological activities described in the "Biological Activities" section below and/or as disclosed for a given Therapeutic protein in Table 1 (column 2).

**[0054]** As used herein, "therapeutic activity" or "activity" may refer to an activity whose effect is consistent with a desirable therapeutic outcome in humans, or to desired effects in non-human mammals or in other species or organisms. Therapeutic activity may be measured *in vivo* or *in vitro*. For example, a desirable effect may be assayed in cell culture. As an example, when EPO is the Therapeutic protein, the effects of EPO on cell proliferation as described in Example 8 may be used as the endpoint for which therapeutic activity is measured. Such *in vitro* or cell culture assays are commonly available for many Therapeutic proteins as described in the art. Examples of assays include, but are not limited to those described herein in the Examples section or in the "Exemplary Activity-Assay" column (column 3) of Table 1.

**[0055]** Therapeutic proteins corresponding to a Therapeutic protein portion of an albumin fusion protein of the invention, such as cell surface and secretory proteins, are often modified by the attachment of one or more oligosaccharide groups. The modification, referred to as glycosylation, can dramatically affect the physical properties of proteins and can be important in protein stability, secretion, and localization. Glycosylation occurs at specific

locations along the polypeptide backbone. There are usually two major types of glycosylation: glycosylation characterized by O-linked oligosaccharides, which are attached to serine or threonine residues; and glycosylation characterized by N-linked oligosaccharides, which are attached to asparagine residues in an Asn-X-Ser or Asn-X-Thr sequence, where X can be any amino acid except proline. N-acetylneuramic acid (also known as sialic acid) is usually the terminal residue of both N-linked and O-linked oligosaccharides. Variables such as protein structure and cell type influence the number and nature of the carbohydrate units within the chains at different glycosylation sites. Glycosylation isomers are also common at the same site within a given cell type.

**[0056]** For example, several types of human interferon are glycosylated. Natural human interferon- $\alpha 2$  is O-glycosylated at threonine 106, and N-glycosylation occurs at asparagine 72 in interferon- $\alpha 14$  (Adolf *et al.*, J. Biochem 276:511 (1991); Nyman TA *et al.*, J. Biochem 329:295 (1998)). The oligosaccharides at asparagine 80 in natural interferon- $\beta 1\alpha$  may play an important factor in the solubility and stability of the protein, but may not be essential for its biological activity. This permits the production of an unglycosylated analog (interferon- $\beta 1b$ ) engineered with sequence modifications to enhance stability (Hosoi *et al.*, J. Interferon Res. 8:375 (1988; Karpusas *et al.*, Cell Mol Life Sci 54:1203 (1998); Knight, J. Interferon Res. 2:421 (1982); Runkel *et al.*, Pharm Res 15:641 (1998); Lin, Dev. Biol. Stand. 96:97 (1998)). Interferon- $\gamma$  contains two N-linked oligosaccharide chains at positions 25 and 97, both important for the efficient formation of the bioactive recombinant protein, and having an influence on the pharmacokinetic properties of the protein (Sareneva *et al.*, Eur. J. Biochem 242:191 (1996); Sareneva *et al.*, Biochem J. 303:831 (1994); Sareneva *et al.*, J. Interferon Res. 13:267 (1993)). Mixed O-linked and N-linked glycosylation also occurs, for example in human erythropoietin, N-linked glycosylation occurs at asparagine residues located at positions 24, 38 and 83 while O-linked glycosylation occurs at a serine residue located at position 126 (Lai *et al.*, J. Biol. Chem. 261:3116 (1986); Broudy *et al.*, Arch. Biochem. Biophys. 265:329 (1988)).

**[0057]** Glycosylation of EPO albumin fusion proteins may influence the activity and/or stability of the EPO albumin fusion proteins. The EPO portion of the albumin fusion protein may contain 3 N-linked sites for glycosylation, each of which can carry one tetra-antennary structure. When the EPO albumin fusion protein is glycosylated, the half-life of the molecule may be increased. In one embodiment, the EPO albumin fusion protein is

glycosylated. In another embodiment, the EPO albumin fusion protein is hyperglycosylated.

**[0058]** One type of sugar commonly found in oligosaccharides is sialic acid. Each tetra-antennary structure of the N-linked glycosylation sites of EPO may carry four sialic acid residues. Accordingly, in a preferred embodiment, the EPO albumin fusion protein is glycosylated with a carbohydrate group containing sialic acid. In an additional embodiment, the EPO albumin fusion protein comprises a fully sialylated EPO protein containing four sialic acid residues per tetra-antennary structure per site with a molar ratio of sialic acid to protein 12:1 or greater. In alternative embodiments, the EPO albumin fusion protein comprises a hypersialylated EPO protein wherein one, two, or three sialic acid residues are attached at each tetra-antennary structure per site with a molar ratio of sialic acid to protein less than 12:1.

**[0059]** Two types of sialic acid that may be used in the sialylation of the EPO albumin fusion protein are N-acetylneuraminic acid (Neu5Ac) or N-glycolylneuraminic acid (Neu5Gc). In a preferred embodiment, hypersialylated EPO albumin fusion proteins contain Neu5Ac. More preferably, the total sialic acid content of hypersialylated EPO albumin fusion proteins is at least 97% Neu5Ac. Most preferred are EPO albumin fusion protein structures with little or no Neu5Gc.

**[0060]** Preferably, the albumin EPO fusion protein has at least 4 moles of sialylation, and more preferably, at least 8-9 moles of sialylation. An additional embodiment comprises an albumin EPO fusion protein with 4 moles of sialylation, 5 moles of sialylation, 6 moles of sialylation, 7 moles of sialylation, 8-9 moles of sialylation, 8 moles of sialylation, 9 moles of sialylation, 10 moles of sialylation, 11 moles of sialylation, or 12 moles of sialylation.

**[0061]** The degree of sialylation of a protein changes the charge of the protein and its retention time on a chromatography column. Therefore, certain chromatography steps used in the purification process may be used to monitor or enrich for hypersialylated EPO albumin fusion proteins. In a preferred embodiment, the amount of sialylation may be monitored by HPLC chromatography. In an additional embodiment, steps in the purification process of EPO albumin fusions may be used to enrich for hypersialylated EPO albumin fusion proteins. In a preferred embodiment the purification steps that may be used to enrich for hypersialylated EPO albumin fusion proteins comprise the butyl-sepharose FF purification step to remove virus particles by high ammonium salt and the hydroxyapatite chromatography at pH 6.8 for the final purification step.

**[0062]** Therapeutic proteins corresponding to a Therapeutic protein portion of an

albumin fusion protein of the invention, as well as analogs and variants thereof, may be modified so that glycosylation at one or more sites is altered as a result of manipulation(s) of their nucleic acid sequence, by the host cell in which they are expressed, or due to other conditions of their expression. For example, glycosylation isomers may be produced by abolishing or introducing glycosylation sites, *e.g.*, by substitution or deletion of amino acid residues, such as substitution of glutamine for asparagine, or unglycosylated recombinant proteins may be produced by expressing the proteins in host cells that will not glycosylate them, *e.g.* in *E. coli* or glycosylation-deficient yeast. These approaches are described in more detail below and are known in the art.

**[0063]** Therapeutic proteins, particularly those disclosed in Table 1, and their nucleic acid and amino acid sequences are well known in the art and available in public databases such as Chemical Abstracts Services Databases (*e.g.*, the CAS Registry), GenBank, and subscription provided databases such as GenSeq (*e.g.*, Derwent). Exemplary nucleotide sequences of Therapeutic proteins which may be used to derive a polynucleotide of the invention are shown in column 7, "SEQ ID NO:X," of Table 2. Sequences shown as SEQ ID NO:X may be a wild type polynucleotide sequence encoding a given Therapeutic protein (*e.g.*, either full length or mature), or in some instances the sequence may be a variant of said wild type polynucleotide sequence (*e.g.*, a polynucleotide which encodes the wild type Therapeutic protein, wherein the DNA sequence of said polynucleotide has been optimized, for example, for expression in a particular species; or a polynucleotide encoding a variant of the wild type Therapeutic protein (*i.e.*, a site directed mutant; an allelic variant)). It is well within the ability of the skilled artisan to use the sequence shown as SEQ ID NO:X to derive the construct described in the same row. For example, if SEQ ID NO:X corresponds to a full length protein, but only a portion of that protein is used to generate the specific CID, it is within the skill of the art to rely on molecular biology techniques, such as PCR, to amplify the specific fragment and clone it into the appropriate vector.

**[0064]** Additional Therapeutic proteins corresponding to a Therapeutic protein portion of an albumin fusion protein of the invention include, but are not limited to, one or more of the Therapeutic proteins or peptides disclosed in the "Therapeutic Protein X" column of Table 1 (column 1), or fragment or variable thereof.

**[0065]** Table 1 provides a non-exhaustive list of Therapeutic proteins that correspond to a Therapeutic protein portion of an albumin fusion protein of the invention, or an albumin fusion protein encoded by a polynucleotide of the invention. The first column, "Therapeutic

Protein X,” discloses Therapeutic protein molecules that may be followed by parentheses containing scientific and brand names of proteins that comprise, or alternatively consist of, that Therapeutic protein molecule or a fragment or variant thereof. “Therapeutic protein X” as used herein may refer either to an individual Therapeutic protein molecule, or to the entire group of Therapeutic proteins associated with a given Therapeutic protein molecule disclosed in this column. The “Biological activity” column (column 2) describes Biological activities associated with the Therapeutic protein molecule. Column 3, “Exemplary Activity Assay,” provides references that describe assays which may be used to test the therapeutic and/or biological activity of a Therapeutic protein:X or an albumin fusion protein comprising a Therapeutic protein X (or fragment thereof) portion. Each of the references cited in the “Exemplary Activity Assay” column are herein incorporated by reference in their entireties, particularly with respect to the description of the respective activity assay described in the reference (see Methods section therein, for example) for assaying the corresponding biological activity set forth in the "Biological Activity" column of Table 1. The fourth column, “Preferred Indication: Y,” describes disease, disorders, and/or conditions that may be treated, prevented, diagnosed, and/or ameliorated by Therapeutic protein X or an albumin fusion protein comprising a Therapeutic protein X (or fragment thereof) portion. The “Construct ID” column (column 5) provides a link to an exemplary albumin fusion construct disclosed in Table 2 which encodes an albumin fusion protein comprising, or alternatively consisting of the referenced Therapeutic Protein X (or fragment thereof) portion.

**Table 1**

| Therapeutic Protein:X  | Biological Activity   | Exemplary Activity Assay   | Preferred Indication: Y  | Construct ID  | Therapeutic Protein:Z                              |
|--|---|--|--|---|--|
| EPO<br>(Erythropoietin;<br>Epoetin alfa;<br>Epoetin beta;<br>Gene-activated<br>erythropoietin;<br>Darbepoetin-<br>alpha; NESP;<br>Epogen; Procrit;<br>Eprex; Erypo;<br>Espo; Epoimmun;<br>EPOGIN;<br>NEORECORMO<br>N; HEMOLINK;<br>Dynepo;<br>ARANESP) | Stimulates cellular differentiation of bone-marrow stem cells at an early stage of erythropoiesis; accelerates the proliferation and maturation of terminally differentiating cells into erythrocytes; and modulates the level of circulating erythrocytes. | Cell proliferation assay using a erythroleukemic cell line TF-1. (Kitamura et al. 1989 J.Cell. Physiol. 140:323) | Anemia; Anemia in Renal Disease; Anemia in Oncology Patients; Bleeding Disorders; Chronic Renal Failure; Chronic Renal Failure in Pre-Dialysis Patients; Renal Disease; End-Stage Renal Disease; End-Stage Renal Disease in Dialysis Patients; Chemotherapy in Cancer Patients; Anemia in zidovudine-treated HIV patients; Anemia in zidovudine-treated patients; Anemia in HIV patients; Anemia in premature infants; Surgical patients (pre and/or post surgery); Surgical patients (pre and/or post surgery) who are anemic; Surgical patients (pre and/or post surgery) who are undergoing elective surgery; Surgical patients (pre and/or post surgery) who are undergoing elective, non-cardiac surgery; Surgical patients (pre and/or post surgery) who are undergoing elective, non-cardiac, non-vascular surgery; Surgical patients (pre and/or post surgery) who are undergoing elective, non-vascular surgery; Surgical patients (pre and/or post surgery) who are undergoing cardiac and/or vascular surgery; Aplastic anemia; Refractory anemia; Anemia in Inflammatory Bowel Disease; Refractory anemia in Inflammatory Bowel Disease; Transfusion avoidance; Transfusion avoidance for surgical patients; Transfusion avoidance for elective surgical patients; Transfusion avoidance for elective orthopedic surgical patients; Patients who want to Increase Red Blood Cells. | 1772, 1774, 1781, 1783, 1793, 1794, 1925, 1926, 1966, 1969, 1980, 1981, 1994, 1995, 1996, 1997, 2047, 2102, 2283, 2284, 2287, 2289, 2294, 2298, 2310, 2311, 2325, 2326, 2344, 2363, 2373, 2387, 2414, 2441, 2603, 2604, 2605, 3194, 3195, 3196, | See Table 2, SEQ ID NO:Z for particular construct. |
| G-CSF<br>(Granulocyte colony-  | Stimulates the proliferation and differentiation of the   | Proliferation of murine NFS-60 cells (Weinstein et al, Proc Natl Acad Sci U S                                    | Chemoprotection; Adjunct to Chemotherapy; Inflammatory disorders; Cancer; Leukemia; Myelocytic leukemia; Neutropenia, Primary  | 1642, 1643, 2363, 2373, 2387, 2414, 2441, 2702, 2637, 2700, 2701, 2703,   | See Table 2, SEQ ID NO:Z for particular            |



**Table 1**

| <b>Therapeutic Protein:X</b>   | <b>Biological Activity</b>  | <b>Exemplary Activity Assay</b>  | <b>Preferred Indication: Y</b>   | <b>Construct ID</b>              | <b>Therapeutic Protein:Z</b>                                |
|--|---|--|--|----------------------------------|---|
| stimulating factor;<br>Granulokine;<br>KRN 8601;<br>Filgrastim;<br>Lenograstim;<br>Meograstim;<br>Nartograstim;<br>Neupogen;<br>NOPIA; Gran;<br>GRANOCYTE;<br>Granulokine;<br>Neutrogin; Neu-<br>up; Neutromax)  | progenitor cells for<br>granulocytes and<br>monocytes-macrophages.                            | A 1986; 83, pp5010-4)  | neutropenias (e.g.; Kostmann syndrome);<br>Secondary neutropenia; Prevention of<br>neutropenia; Prevention and treatment of<br>neutropenia in HIV-infected patients; Prevention<br>and treatment of neutropenia associated with<br>chemotherapy; Infections associated with<br>neutropenias; Myelodysplasia; Autoimmune<br>disorders; Psoriasis; Mobilization of<br>hematopoietic progenitor cells; Wound Healing;<br>Autoimmune Disease; Transplants; Bone marrow<br>transplants; Acute myelogenous leukemia;<br>Lymphoma, Non-Hodgkin's lymphoma; Acute<br>lymphoblastic leukemia; Hodgkin's disease;<br>Accelerated myeloid recovery; Glycogen storage<br>disease.  | 2886, 2887, 2888, 2889,<br>2890, | construct.  |
| GM-CSF<br>(Granulocyte-<br>macrophage<br>colony-<br>stimulating factor;<br>rhuGM-CSF; BI<br>61012; Prokine;<br>Molgramostim;<br>Sargramostim;<br>GM-CSF/IL 3<br>fusion;<br>Milodistim;<br>Leucotropin;<br>PROKINE;<br>LEUKOMAX;<br>Interberin;<br>Leukine; Leukine<br>Liquid; Pixykin) | Regulates hematopoietic<br>cell differentiation, gene<br>expression, growth, and<br>function. | Colony Stimulating Assay:<br>Testa, N.G., et al., "Assays<br>for hematopoietic growth<br>factors." Balkwill FR (edt)<br>Cytokines, A practical<br>Approach, pp 229-44; IRL<br>Press Oxford 1991. | Bone Marrow Disorders; Bone marrow<br>transplant; Chemoprotection; Hepatitis C; HIV<br>Infections; Cancer; Lung Cancer; Melanoma;<br>Malignant melanoma; Mycobacterium avium<br>complex; Mycoses; Leukemia; Myeloid<br>Leukemia; Infections; Neonatal infections;<br>Neutropenia; Mucositis; Oral Mucositis; Prostate<br>Cancer; Stem Cell Mobilization; Vaccine<br>Adjuvant; Ulcers (such as Diabetic, Venous<br>Stasis, or Pressure Ulcers); Prevention of<br>neutropenia; Acute myelogenous leukemia;<br>Hematopoietic progenitor cell mobilization;<br>Lymphoma; Non-Hodgkin's lymphoma; Acute<br>Lymphoblastic Leukemia; Hodgkin's disease;<br>Accelerated myeloid recovery; Transplant<br>Rejection; Xenotransplant Rejection. | 1697, 1699, 2066, and 2067.      | See Table 2,<br>SEQ ID NO:Z<br>for particular<br>construct. |

**Table 1**

| <b>Therapeutic Protein:X</b>  | <b>Biological Activity</b>   | <b>Exemplary Activity Assay</b>   | <b>Preferred Indication: Y</b>  | <b>Construct ID</b>  | <b>Therapeutic Protein:Z</b>                       |
|---|--|---|---|--|--|
| Human growth hormone (Pegvisamont; Somatrem; Somatropin; TROVERT; PROTROPIN; BIO-TROPIN; HUMATROPE; NUTROPIN; NUTROPIN AQ; NUTROPHIN; NORDITROPIN; GENOTROPIN; SAIZEN; SEROSTIM)                          | Binds to two GHR molecules and Induces signal transduction through receptor dimerization | Ba/F3-hGHR proliferation assay, a novel specific bioassay for serum human growth hormone. J Clin Endocrinol Metab 2000 Nov;85(11):4274-9<br>Plasma growth hormone (GH) immunoassay and tibial bioassay, Appl Physiol 2000 Dec;89(6):2174-8<br>Growth hormone (hGH) receptor mediated cell mediated proliferation, Growth Horm IGF Res 2000 Oct;10(5):248-55<br>International standard for growth hormone, Horm Res 1999;51 Suppl 1:7-12 | Acromegaly; Growth failure; Growth hormone replacement; Growth hormone deficiency; Pediatric Growth Hormone Deficiency; Adult Growth Hormone Deficiency; Idiopathic Growth Hormone Deficiency; Growth retardation; Prader-Willi Syndrome; Prader-Willi Syndrome in children 2 years or older; Growth deficiencies; Growth failure associated with chronic renal insufficiency; Osteoporosis; Postmenopausal osteoporosis; Osteopenia, Osteoclastogenesis; burns; Cachexia; Cancer Cachexia; Dwarfism; Metabolic Disorders; Obesity; Renal failure; Turner's Syndrome; Fibromyalgia; Fracture treatment; Frailty, AIDS wasting; Muscle Wasting; Short Stature; Diagnostic Agents; Female Infertility; lipodystrophy. | 3163, 2983,  | See Table 2, SEQ ID NO:Z for particular construct. |
| Insulin (Human insulin; Insulin aspart; Insulin Glargine; Insulin lispro; Lys-B28 Pro-B29; lyspro; LY 275585; diarginylinsulin; Des-B26-B30-insulin-B25-amide; Insulin detemir; LABI; NOVOLIN; NOVORAPID; | Stimulates glucose uptake and promotes glycogenesis and lipogenesis.                     | Insulin activity may be assayed in vitro using a [3-H]-glucose uptake assay. (J Biol Chem 1999 Oct 22; 274(43):30864-30873).  | Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X.   | 2250, 2255, 2276, 2278, 2656, 2668, 2669, 2671, 2821, 2822, 2832, 2877, 2878, 2882, 2885, 2891, 2897, 2930, 2931, 2942, 2986, 3025, 3133, 3134, 3197, 3198, 2726, 2727, 2784, 2789 | See Table 2, SEQ ID NO:Z for particular construct. |

**Table 1**

| Therapeutic Protein:X   | Biological Activity | Exemplary Activity Assay     | Preferred Indication:Y                       | Construct ID            | Therapeutic Protein:Z |
|---|---------------------|------------------------------|--|-------------------------|-----------------------|
| HUMULIN;<br>NOVOMIX 30;<br>VELOSULIN;<br>NOVOLOG;<br>LANTUS;<br>ILETIN;<br>HUMALOG;<br>MACRULIN;<br>EXUBRA;<br>INSUMAN;<br>ORALIN;<br>ORALGEN;<br>HUMAHALE;<br>HUMAHALIN) |                     |                              |  |                         |                       |
| Interferon alfa   | Confers a range of  | Anti-viral assay; Rubinstein | Viral infections; HIV Infections; Hepatitis; | 2249, 2343, 2366, 2381, | See Table 2,          |

**Table 1**

| Therapeutic Protein:X   | Biological Activity   | Exemplary Activity Assay  | Preferred Indication: Y   | Construct ID          | Therapeutic Protein:Z                 |
|---|---|---|---|-----------------------|---------------------------------------|
| (Interferon alfa-2b; recombinant; Interferon alfa-n1; Interferon alfa-n3; Peginterferon alfa-2b; Ribavirin and interferon alfa-2b; Interferon alfacon-1; interferon consensus; YM 643; C1FN; interferon -alpha consensus; | cellular responses including antiviral, antiproliferative, antitumor and immunomodulatory activities; stimulate production of two enzymes: a protein kinase and an oligoadenylate synthetase. | S, Familletti PC, Pestka S. (1981) Convenient assay for interferons. J. Virol. 37(2):755-8; Anti-proliferation assay: Gao Y, et al (1999) Sensitivity of an epstein-barr virus-positive tumor line, Daudi, to alpha interferon correlates with expression of a GC-rich viral transcript. Mol Cell Biol. 19(11):7305-13. | Chronic Hepatitis; Hepatitis B; Chronic Hepatitis B; Hepatitis C; Chronic Hepatitis C; Hepatitis D; Chronic Hepatitis D; Human Papillomavirus; Herpes Simplex Virus Infection; External Condylomata Acuminata; HIV; HIV Infection; Oncology; Cancer; Solid Tumors; Melanoma; Malignant Melanoma; Renal Cancer (e.g., Renal Cell Carcinoma); Lung Cancer (e.g., Non-Small Cell Lung Cancer or Small Cell Lung Cancer) Colon Cancer; Breast Cancer; Liver Cancer; Prostate Cancer; Bladder Cancer; Gastric Cancer; Sarcoma; AIDS-Related Kaposi's Sarcoma; Lymphoma; T Cell Lymphoma; Cutaneous T-Cell Lymphoma; Non-Hodgkin's Lymphoma; Brain Cancer; Glioma; Glioblastoma Multiforme; | 2382, 2410, and 3165. | SEQ ID NO:Z for particular construct. |

**Table 1**

| Therapeutic Protein:X   | Biological Activity | Exemplary Activity Assay | Preferred Indication: Y   | Construct ID | Therapeutic Protein:Z |
|---|---------------------|--------------------------|---|--------------|-----------------------|
| recombinant methionyl consensus interferon; recombinant consensus interferon; CGP 35269; RO 253036; RO 258310; INTRON A; PEG-INTRON; OIF; OMNIFERON; PEG-OMNIFERON; |                     |                          | Cervical Dysplasia; Leukemia; Preleukemia; Bone Marrow Disorders; Bone Disorders; Hairy Cell Leukemia; Chronic Myelogenous Leukemia; Hematological Malignancies; Hematological Disorders; Multiple Myeloma; Bacterial Infections; Chemoprotection; Thrombocytopenia; Multiple Sclerosis; Pulmonary Fibrosis; Age-Related Macular Degeneration; Macular Degeneration; Crohn's Disease; Neurological Disorders; Arthritis; Rheumatoid Arthritis; Ulcerative Colitis; Osteoporosis, Osteopenia, Osteoclastogenesis; Fibromyalgia; Sjogren's Syndrome; Chronic Fatigue Syndrome; Fever; Hemorrhagic Fever; Viral Hemorrhagic Fevers; Hyperglycemia; Diabetes; Diabetes Insipidus; |              |                       |

**Table 1**

| Therapeutic Protein:X   | Biological Activity | Exemplary Activity Assay | Preferred Indication: Y  | Construct ID | Therapeutic Protein:Z |
|---|---------------------|--------------------------|--|--------------|-----------------------|
| VELDONA;<br>PEG-<br>REBETRON;<br>ROFERON A;<br>WELLFERON;<br>ALFERON<br>N/LDO;<br>REBETRON;<br>ALTEMOL;<br>VIRAFERONPE<br>G; PEGASYS;<br>VIRAFERON;<br>VIRAFON;<br>AMPLIGEN;<br>INFERGEN; |                     |                          | Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin-dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X. |              |                       |

**Table 1**

| Therapeutic Protein:X  | Biological Activity   | Exemplary Activity Assay  | Preferred Indication: Y   | Construct ID                                   | Therapeutic Protein:Z                              |
|--|---|---|---|--|--|
| INFAREX;<br>ORAGEN)  |   |   |   |  |  |
| Calcitonin (Salmon Calcitonin (Salcatonin); Calcitonin human-salmon hybrid; Forcalcitonin; Fortical; Calcitonin; Calcitonina Almirall; Calcitonina Hubber; | Regulates levels of calcium and phosphate in serum; causes a reduction in serum calcium--an effect opposite to that of human parathyroid hormone. | Hypocalcemic Rat Bioassay, bone resorbing assay and the pit assay, CT receptor binding assay, CAMP stimulation assay: J Bone Miner Res 1999 Aug;14(8):1425-31 | Bone Disorders; Fracture prevention; Hypercalcemia; Malignant hypercalcemia; Osteoporosis; Paget's disease; Osteopenia, Osteoclastogenesis; osteolysis; osteomyelitis; osteonecrosis; periodontal bone loss; osteoarthritis; rheumatoid arthritis; osteopetrosis; periodontal, lytic, or metastatic bone disease; osteoclast differentiation inhibition; bone disorders; bone healing and regeneration. | 1833, 1834, 1835, 1836, 2447, 2513, 2806, 2915 | See Table 2, SEQ ID NO:Z for particular construct. |

**Table 1**

| Therapeutic Protein:X   | Biological Activity   | Exemplary Activity Assay     | Preferred Indication: Y                     | Construct ID            | Therapeutic Protein:Z |
|---|-----------------------|------------------------------|---|-------------------------|-----------------------|
| Calcimar;Calsynar; Calogen; Miacalcic; Miacalcin; SB205614; Macritonin; Cibacalcin; Cibacalcina; Cibacalcine; Salmocalcin; PowderJect Calcitonin (CAS-21215-62-3) |                       |                              |   |                         |                       |
| Interferon beta   | Modulates MHC antigen | Anti-viral assay; Rubinstein | Multiple Sclerosis; Oncology; Cancer; Solid | 1778, 1779, 2011, 2013, | See Table 2,          |



**Table 1**

| <b>Therapeutic Protein:X</b>  | <b>Biological Activity</b>   | <b>Exemplary Activity Assay</b>   | <b>Preferred Indication: Y</b>   | <b>Construct ID</b>                       | <b>Therapeutic Protein:Z</b>          |
|---|--|---|--|---|---------------------------------------|
| (Interferon beta-1a; Interferon beta 1b; Interferon-beta-serine; SH 579; ZK 157046; BCDF; beta-2 IF; Interferon-beta-2; rhIL-6; SJ0031; DL 8234; FERON; IFNbeta; BETASERON; AVONEX; REBIF; BETAFERON; | expression, NK cell activity and IFNg production and IL12 production in monocytes. | S. Familletti PC, Pestka S. (1981) Convenient assay for interferons. J. Virol. 37(2):755-8; Anti-proliferation assay: Gao Y, et al (1999) Sensitivity of an Epstein-Barr virus-positive tumor line, Daudi, to alpha interferon correlates with expression of a GC-rich viral transcript. Mol Cell Biol. 19(11):7305-13. | Tumors; Melanoma; Malignant Melanoma; Renal Cancer (e.g., Renal Cell Carcinoma); Lung Cancer (e.g., Non-Small Cell Lung Cancer or Small Cell Lung Cancer) Colon Cancer; Breast Cancer; Liver Cancer; Prostate Cancer; Bladder Cancer; Gastric Cancer; Sarcoma; AIDS-Related Kaposi's Sarcoma; Lymphoma; T Cell Lymphoma; Cutaneous T-Cell Lymphoma; Non-Hodgkin's Lymphoma; Brain Cancer; Glioma; Glioblastoma Multiforme; Cervical Dysplasia; Leukemia; Preleukemia; Bone Marrow Disorders; Bone Disorders; Hairy Cell Leukemia; Chronic Myelogenous Leukemia; Hematological Malignancies; Hematological Disorders; Multiple Myeloma; Bacterial Infections; | 2053, 2054, 2492, 2580, 2795, 2796, 2797. | SEQ ID NO:Z for particular construct. |

**Table 1**

| Therapeutic Protein:X | Biological Activity | Exemplary Activity Assay | Preferred Indication: Y   | Construct ID | Therapeutic Protein:Z |
|-----------------------|---------------------|--------------------------|---|--------------|-----------------------|
| SIGOSIX)              |                     |                          | Chemoprotection; Thrombocytopenia; Viral infections; HIV Infections; Hepatitis; Chronic Hepatitis B; Hepatitis C; Chronic Hepatitis C; Hepatitis D; Chronic Hepatitis D; Human Papillomavirus; Herpes Simplex Virus Infection; External Condylomata Acuminata; HIV; HIV Infection; Pulmonary Fibrosis; Age-Related Macular Degeneration; Macular Degeneration; Crohn's Disease; Neurological Disorders; Arthritis; Rheumatoid Arthritis; Ulcerative Colitis; Osteoporosis, Osteopenia, Osteoclastogenesis; Fibromyalgia; Sjogren's Syndrome; Chronic Fatigue Syndrome; Fever; Hemorrhagic Fever; Viral Hemorrhagic Fevers; Hyperglycemia; |              | /                     |

**Table 1**

| Therapeutic Protein:X            | Biological Activity                             | Exemplary Activity Assay                      | Preferred Indication: Y  | Construct ID   | Therapeutic Protein:Z    |
|----------------------------------|---|---|--|----------------|--------------------------|
|                                  |   |   | Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X. |                |                          |
| Growth hormone releasing factor; | Acts on the anterior pituitary to stimulate the | Growth hormone-releasing peptides (GHRPs) are | Acromegaly; Growth failure; Growth hormone replacement; Growth hormone deficiency;   | 1747 and 1748. | See Table 2, SEQ ID NO:Z |

**Table 1**

| Therapeutic Protein:X   | Biological Activity   | Exemplary Activity Assay   | Preferred Indication:Y   | Construct ID            | Therapeutic Protein:Z     |
|---|---|--|--|-------------------------|---------------------------|
| Growth hormone releasing hormone (Sermorelin acetate; Pralmorelin; Somatostatin; Somatoliberin; Geref; Gerel; Groliberin) | production and secretion of growth hormone and exert a trophic effect on the gland. | known to release growth hormone (GH) in vivo and in vitro by a direct action on receptors in anterior pituitary cells. Biological activity can be measured in cell lines expressing growth hormone releasing factor receptor (Mol Endocrinol 1992 Oct;6(10):1734-44, Molecular Endocrinology, Vol 7, 77-84). | Pediatric Growth Hormone Deficiency; Adult Growth Hormone Deficiency; Idiopathic Growth Hormone Deficiency; Growth retardation; Prader-Willi Syndrome; Prader-Willi Syndrome in children 2 years or older; Growth deficiencies; Growth failure associated with chronic renal insufficiency; Osteoporosis; Osteopenia, Osteoclastogenesis; Postmenopausal osteoporosis; burns; Cachexia; Cancer Cachexia; Dwarfism; Metabolic Disorders; Obesity; Renal failure; Turner's Syndrome; Fibromyalgia; Fracture treatment; Frailty, AIDS wasting; Muscle Wasting; Short Stature; Diagnostic Agents; Female Infertility; lipodystrophy. |                         | for particular construct. |
| IL-2  | Promotes the growth of B  | T cell proliferation assay   | Cancer; Solid Tumors; Metastatic Renal Cell  | 1757, 1758, 1812, 1813, | See Table 2,              |

**Table 1**

| Therapeutic Protein:X   | Biological Activity   | Exemplary Activity Assay  | Preferred Indication: Y   | Construct ID                | Therapeutic Protein:Z                 |
|---|---|---|---|-----------------------------|---------------------------------------|
| (Aldesleukin; interleukin-2 fusion toxin; T cell growth factor; PROLEUKIN; IMMUNACE; CELEUK; ONCOLIPIN 2; MACROLIN) | and T cells and augments NK cell and CTL cell killing activity. | "Biological activity of recombinant human interleukin-2 produced in Escherichia coli." Science 223: 1412-1415, 1984.<br>natural killer (NK) cell and CTL cytotoxicity assay<br>"Control of homeostasis of CD8+ memory T cells by opposing cytokines. Science 288: 675-678, 2000; CTLL-2 Proliferation : Gillis et al (1978) J. Immunol. 120, 2027 | Carcinoma; Metastatic Melanoma; Malignant Melanoma; Melanoma; Renal Cell Carcinoma; Renal Cancer; Lung Cancer (e.g., Non-Small Cell Lung Cancer or Small Cell Lung Cancer); Colon Cancer; Breast Cancer; Liver Cancer; Leukemia; Preleukemia; Hematological Malignancies; Hematological Disorders; Acute Myeloid Leukemia; Melanoma; Malignant Melanoma; Non-Hodgkin's Lymphoma; Ovarian Cancer; Prostate Cancer; Brain Cancer; Glioma; Glioblastoma Multiforme; Hepatitis; Hepatitis C; Lymphoma; HIV Infection (AIDS); Inflammatory Bowel Disorders; Kaposi's Sarcoma; Multiple Sclerosis; Arthritis; Rheumatoid Arthritis; Transplant Rejection; | 1952, 1954, 2030, and 2031. | SEQ ID NO:Z for particular construct. |

**Table 1**

| Therapeutic Protein:X   | Biological Activity   | Exemplary Activity Assay  | Preferred Indication: Y   | Construct ID  | Therapeutic Protein:Z                              |
|---|---|---|---|---|--|
| Parathyroid hormone; parathyrin (PTH; Ostabolin; ALX1-11; hPTH 1-34; LY 333334; MN 10T; parathyroid hormone (1-31); FORTEO; PARATHAR) | Acts in conjunction with calcitonin to control calcium and phosphate metabolism; elevates blood calcium level; stimulates the activity of osteocytes; enhances absorption of Ca <sup>++</sup> /Pi from small intestine into blood; promotes reabsorption of Ca <sup>++</sup> and inhibits Pi by kidney tubules. | Adenylyl cyclase stimulation in rat osteosarcoma cells, ovariectomized rat model of osteoporosis: IUBMB Life 2000 Feb;49(2):131-5                               | Diabetes; Type 1 Diabetes Mellitus; Type 2 Diabetes.<br><br>Bone Disorders; Fracture prevention; Hypercalcemia; Malignant hypercalcemia; Osteoporosis; Paget's disease; Osteopenia, Osteoclastogenesis; osteolysis; osteomyelitis; osteonecrosis; periodontal bone loss; osteoarthritis; rheumatoid arthritis; osteopetrosis; periodontal, lytic, or metastatic bone disease; osteoclast differentiation inhibition; bone disorders; bone healing and regeneration.   | 1749, 1750, 1853, 1854, 1889, 1906, 1907, 1914, 1932, 1938, 1941, 1949, 2021, 2022, 2023, 2428, 2714, 2791, 2965, 2966. | See Table 2, SEQ ID NO:Z for particular construct. |
| Resistin  | Mediates insulin resistance in Type II diabetes; inhibits insulin-stimulated glucose uptake   | Ability of resistin to influence type II diabetes can be determined using assays known in the art: Pontoglio et al., J Clin Invest 1998 May 15;101(10):2215-22. | Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X. | 2295, 2296, 2297, 2300, and 2309.   | See Table 2, SEQ ID NO:Z for particular construct. |
| TR6 (DcR3; Decoy Receptor 3; FASTR)   | Inhibits Fas Ligand and AIM-2 (TL5, LIGHT) mediated apoptosis.  | Cellular apoptosis can be measured by annexin staining, TUNEL staining, measurement of caspase levels. Inhibition of cell growth can also be directly           | Fas Ligand or LIGHT induced apoptotic disorders: hepatitis; liver failure (including fulminant liver failure); graft versus host disease; graft rejection; myelodysplastic syndrome; renal failure; insulin dependent diabetes mellitus; rheumatoid arthritis;  | 1520, 1537, 1545, 1546, 1568, 1570, 1622, 1623, 1645, 1700, 1702, 1703, 1704, 1891, 1892, 1912, and 1913.               | See Table 2, SEQ ID NO:Z for particular construct. |

**Table 1**

| Therapeutic Protein:X  | Biological Activity   | Exemplary Activity Assay   | Preferred Indication:Y  | Construct ID | Therapeutic Protein:Z                              |
|--|---|--|---|--------------|--|
|  |   | measured, for example by ALOMAR Blue staining. Assay refs: cytotoxicity assay on human fibrosarcoma (Epsevik and Nissen-Meyer, 1986, J. Immunol. methods).         | inflammatory bowel disease; autoimmune disease; toxic epidermal necrolysis; multiple sclerosis.                                     |              |  |
| DeCAF (D-SLAM; BCM-like membrane protein; BLAME (B lymphocyte activator macrophage | Inhibits proliferation and differentiation of B cells; Antagonize BLyS activity | DeCAF activity can be determined using assays known in the art, such as for example, those described in Examples 32-33 of International Publication No. WO0111046. | B cell and/or T cell mediated immune disorders; Immunodeficiency (e.g., Common Variable Immunodeficiency, Selective IgA Deficiency) | 1657.        | See Table 2, SEQ ID NO:Z for particular construct. |

**Table 1**

| Therapeutic Protein:X  | Biological Activity   | Exemplary Activity Assay   | Preferred Indication: Y  | Construct ID                  | Therapeutic Protein:Z                              |
|--|---|--|--|-------------------------------|--|
| expressed))  |   |  |  |                               |  |
| BlyS (B Lymphocyte Stimulator; Neutrokin alpha; TL7; BAFF; TALL-1; THANK; radiolabeled BlyS) | Promotes proliferation, differentiation and survival of B cells; Promotes immunoglobulin production by B cells. | BlyS activity can be determined using assays known in the art, such as, for example, the costimulatory proliferation assay and other assays disclosed by Moore et al., 1999, Science, 285(5425):260-3.   | B cell and/or T cell mediated immune disorders, particularly immune system disorders associated with low B cell numbers or low serum immunoglobulin; Immunodeficiency (e.g., Common Variable Immunodeficiency, Selective IgA Deficiency). Radiolabeled forms: lymphoma, non-Hodgkins lymphoma, chronic lymphocytic leukemia, multiple myeloma.                             | 1680, 2095, and 2096.         | See Table 2, SEQ ID NO:Z for particular construct. |
| Anti-BlyS single chain antibody (sc Fv1116A01, scFvI050B11, scFvI006D08) and others.         | Agonize or antagonize BlyS activity.  | BlyS agonist or antagonist activity can be determined using assays known in the art, such as, for example, a modified version the costimulatory proliferation assay disclosed by Moore et al., 1999, Science, 285(5425):260-3, in which BlyS is mixed or preincubated with the anti-BlyS antibody prior to being applied to the responder B lymphocytes. | B cell and/or T cell mediated immune disorders; Autoimmune disorders, particularly autoimmune diseases associated with the production of autoantibodies; Rheumatoid Arthritis, Systemic Lupus Erythematosus; Sjögren's Syndrome, cancers expressing BlyS as an autocrine growth factor, e.g. certain chronic lymphocytic leukemias.  | 1821, 1956, 2501, 2502, 2638. | See Table 2, SEQ ID NO:Z for particular construct. |
| MPIF-1 (Myeloid Progenitor Inhibitory Factor; CK beta-8; Mirostipen)                         | Inhibits myeloid progenitor cells; and activates monocytes  | MPIF-1 activity can be measured using the myeloprotection assay and chemotaxis assay described in US patent 6,001,606.   | Chemoprotection; Adjunct to Chemotherapy; Inflammatory disorders; Cancer; Leukemia; Myelocytic leukemia; Neutropenia, Primary neutropenias (e.g.; Kostmann syndrome); Secondary neutropenia; Prevention of neutropenia; Prevention and treatment of neutropenia in HIV-infected patients; Prevention and treatment of neutropenia associated with chemotherapy; Infections | 1681, 3166, 3167, 3168,       | See Table 2, SEQ ID NO:Z for particular construct. |



**Table 1**

| Therapeutic Protein:X                                  | Biological Activity   | Exemplary Activity Assay  | Preferred Indication: Y  | Construct ID | Therapeutic Protein:Z                              |
|--|---|---|--|--------------|--|
|  |   |   | associated with neutropenias; Myelodysplasia; Autoimmune disorders; Psoriasis; Mobilization of hematopoietic progenitor cells; Wound Healing; Autoimmune Disease; Transplants; Bone marrow transplants; Acute myelogenous leukemia; Lymphoma, Non-Hodgkin's lymphoma; Acute lymphoblastic leukemia; Hodgkin's disease; Accelerated myeloid recovery; Glycogen storage disease. |              |  |
| KDI (Keratinocyte Derived Interferon; Interferon Kappa | Inhibits bone marrow proliferation; and shows antiviral activity. | KDI activity can be measured using the antiviral and cell proliferation assays described in Examples 57-63 of International Publication No. | Multiple sclerosis; Hepatitis; Cancer; Viral infections, HIV infections, Leukemia.   | 1746.        | See Table 2, SEQ ID NO:Z for particular construct. |

**Table 1**

| Therapeutic Protein:X   | Biological Activity   | Exemplary Activity Assay  | Preferred Indication: Y   | Construct ID   | Therapeutic Protein:Z                              |
|---|---|---|---|--|--|
| Precursor)  |   | WO0107608.  |   |  |  |
| TNFR2 (p75) (ENBREL)  | Binds both TNFa and TNFb; mediates T-cell proliferation by TNF; reduces signs and structural damage in patients with moderately to severely active rheumatoid arthritis (RA). | T-cell proliferation can be measured using assays known in the art. For example, "Lymphocytes: a practical approach" edited by: SL Rowland, AJ McMichael – chapter 6, pages 138-160 Oxford University Press (2000); and "Current Protocols on CD-ROM" section 3.12 Proliferation Assays for T-cell Function John Wiley & Sons, Inc. (1999). | Autoimmune disease; Rheumatoid Arthritis; Psoriatic arthritis; Still's Disease; Ankylosing Spondylitis; Cardiovascular Diseases; Vasculitis; Wegener's granulomatosis; Amyloidosis; Systemic Lupus Erythematosus, Insulin-Dependent Diabetes Mellitus; Immunodeficiency Disorders; Infection; Inflammation; Inflammatory Bowel Disease; Chrohn's Disease; Psoriasis; AIDS; Graft Rejection; Graft Versus Host Disease.  | 1777 and 1784.   | See Table 2, SEQ ID NO:Z for particular construct. |
| Keratinocyte growth factor 2 (Repifermin; KGF-2; Fibroblast Growth Factor-10; FGF-10) | Stimulates epithelial cell growth.  | KGF-2 activity can be measured using the wound healing assays and epithelial cell proliferation assays described in US patent 6,077,692.  | Stimulate Epithelial Cell Proliferation; Stimulate Basal Keratinocytes; Wound Healing; Stimulate Hair Follicle Production; Healing Of Dermal Wounds. Wound Healing; Eye Tissue Wounds, Dental Tissue Wounds, Oral Cavity Wounds, Diabetic Ulcers, Dermal Ulcers, Cubitus Ulcers, Arterial Ulcers, Venous Stasis Ulcers, Burns Resulting From Heat Exposure Or Chemicals, or Other Abnormal Wound Healing Conditions such as Uremia, Malnutrition, Vitamin Deficiencies or Complications Associated With Systemic Treatment With Steroids, Radiation Therapy or Antineoplastic Drugs or Antimetabolites; Promote Dermal Reestablishment Subsequent To Dermal Loss; Increase the Adherence Of Skin Grafts To A Wound Bed; Stimulate Re-Epithelialization from The Wound Bed; To | 1785, 1786, 1916, 1917, 2498, 2499, 2552, 2553, 2584, 2607, 2608, 2606, 2630 | See Table 2, SEQ ID NO:Z for particular construct. |

**Table 1**

| Therapeutic Protein:X | Biological Activity | Exemplary Activity Assay | Preferred Indication: Y   | Construct ID | Therapeutic Protein:Z |
|-----------------------|---------------------|--------------------------|---|--------------|-----------------------|
|                       |                     |                          | Promote Skin Strength; Improve The Appearance Of Aged Skin; Proliferate Hepatocytes, Lung, Breast, Pancreas, Stomach, Bladder, Small Intestine, Large Intestine; Sebocytes, Hair Follicles, Type II Pneumocytes, Mucin-Producing Goblet Cells, or Other Epithelial Cells, Endothelial Cells, Keratinocytes, or Basal Keratinocytes (and Their Progenitors) Contained Within The Skin, Lung, Liver, Bladder, Eye, Salivary Glands, or Gastrointestinal Tract; Reduce The Side Effects Of Gut Toxicity That Result From Radiation, Chemotherapy Treatments Or Viral Infections; Cytoprotector, especially of the Small Intestine Mucosa or Bladder; Mucositis |              |                       |

**Table 1**

| Therapeutic Protein:X | Biological Activity | Exemplary Activity Assay | Preferred Indication: Y   | Construct ID | Therapeutic Protein:Z |
|-----------------------|---------------------|--------------------------|---|--------------|-----------------------|
|                       |                     |                          | (Mouth Ulcers); Regeneration Of Skin; Full and/or Partial Thickness Skin Defects, including Burns, (e.g., Repopulation Of Hair Follicles, Sweat Glands, And Sebaceous Glands); Psoriasis; Epidermolysis Bullosa; Blisters; Gastric and/or Doudenal Ulcers; Reduce Scarring; Inflammatory Bowel Diseases; Crohn's Disease; Ulcerative Colitis; Gut Toxicity; Lung Damage; Repair Of Alveoli And/or Brochiolar Epithelium; Acute Or Chronic Lung Damage; Emphysema, ARDS; Inhalation Injuries; Hyaline Membrane Diseases; Infant Respiratory Distress Syndrome; Bronchopulmonary Displasia In Premature Infants; Fulminant Liver Failure; |              |                       |

**Table 1**

| Therapeutic Protein:X   | Biological Activity   | Exemplary Activity Assay  | Preferred Indication: Y  | Construct ID                            | Therapeutic Protein:Z                              |
|---|---|---|--|---|--|
|   |   |   | Cirrhosis, Liver Damage caused by Viral Hepatitis and/or Toxic Substances; Diabetes Mellitus; Inflammation.  |   |  |
| TR2 (and TR2sv1, TR2SV2; TNFRSF14; HVEM; Herpes Virus Entry Mediator; ATAR) | Inhibits B cell proliferation, and mediates and inhibits Herpes Simplex Virus (HSV) infection.    | Co-stimulation B-cell proliferation assay and Ig production assay (Moore et al., 1999, Science, 285(5425):260-3.). HSV-1 and HSV-2 Infectivity Assay: International Publication No. WO 97/04658                                     | Herpes; immune disorders; autoimmune disease; graft versus host disease; graft rejection; variable immunodeficiency; immunodeficiency syndromes; cancer. | 1788 and 2129.                          | See Table 2, SEQ ID NO:Z for particular construct. |
| Macrophage derived chemokine, MDC (Ckbeta-13)                               | Chemotactic for monocyte-derived dendritic cells and IL-2-activated natural killer cells.         | Chemokine activities can be determined using assays known in the art: Methods in Molecular Biology, 2000, vol. 138: Chemokine Protocols. Edited by: A.E.I. Proudfoot, T.N.C. Wells, and C.A. Power. © Humana Press Inc., Totowa, NJ | Inflammatory diseases; wound healing; angiogenesis; AIDS infection.  | 1809, 2137, 2474, 2475, 2476, and 2477. | See Table 2, SEQ ID NO:Z for particular construct. |
| HAGDG59 (Retinal short-chain dehydrogenase)                                 | Activates MIP1a release in Dendritic Cells.   | Dendritic cell assays are well known in the art. For example, J. Immunol. 158:2919-2925 (1997); J. Leukoc. Biol. 65:822-828 (1999).   | Immune disorders; cancer; viral infection; inflammation; sepsis; arthritis; asthma.  | 1830 and 1831.                          | See Table 2, SEQ ID NO:Z for particular construct. |
| GnRH (Gonadotropin Releasing Hormone)                                       | Promotes release of follicle-stimulating hormone and luteinizing hormone from anterior pituitary. | GnRH is known to cause the release of follicle stimulating hormone (FSH) and/or luteinizing hormone (LH) in vivo by a direct  | Infertility; Kallmann's syndrome or other forms of hypergonadotropic hypergonadism (failure to go through puberty naturally).                            | 1862 and 1863.                          | See Table 2, SEQ ID NO:Z for particular construct. |

**Table 1**

| Therapeutic Protein:X | Biological Activity                           | Exemplary Activity Assay   | Preferred Indication:Y                  | Construct ID                | Therapeutic Protein:Z                   |
|-----------------------|---|--|---|-----------------------------|---|
|                       |   | action on receptors in anterior pituitary gonadotropes. GnRH activity can be determined by measuring FSH levels in the medium of cultured gonadotropes before and after GnRH supplementation. For example, Baker et al. Biol Reprod 2000 Sep;63(3):865-71. |   |                             |   |
| Teprotide             | Inhibits angiotensin converting enzyme (ACE). | Inhibition of ACE can be determined using assays known in the art. For   | Hypertension; congestive heart failure. | 1866, 1867, 2025, and 2026. | See Table 2, SEQ ID NO:Z for particular |

**Table 1**

| Therapeutic Protein:X                            | Biological Activity  | Exemplary Activity Assay   | Preferred Indication: Y  | Construct ID   | Therapeutic Protein:Z                              |
|--|--|--|--|--|--|
|  |  | example, Anzenbacherova et al., J.Pharma Biomed Anal 2001 Mar; 24(5-6):1151-6.   |  |  | construct.   |
| Human chemokine HCC-1 (ckBeta-1; HWFBD)          | Involved in inflammation, allergy, tissue rejection, viral infection, and tumor biology; enhances proliferation of CD34+ myeloid progenitor cells.   | Chemokine activities can be determined using assays known in the art: Methods in Molecular Biology, 2000, vol. 138: Chemokine Protocols. Edited by: A.E.I. Proudfoot, T.N.C. Wells, and C.A. Power. © Humana Press Inc., Totowa, NJ  | Autoimmune disorders; Immunity; Vascular and Inflammatory disorders; HIV; AIDS; infectious diseases.   | 1933, 1934, 1947, 1948, 1955, 1998, 2355, 2412, 2449, 2837, 2838, 2839, 2840, 2841, 2842, 2843, 2844, 2845, 2849, 2947, 3066, 3105, 3124, 3125, 3139, 3152, 3153, 3154, 3155, 3156, 3169, 3170, 3202, 3203, 3204, 3205, 3206, 3207, 3272 | See Table 2, SEQ ID NO:Z for particular construct. |
| ACE2 inhibitor (DX512)                           | Inhibits production of angiotensin II which induces aldosterone production, arteriolar smooth muscle vasoconstriction, and proliferation of cardiac fibroblasts, Induces angiogenesis; an enzyme that converts angiotensin I to angiotensin I-9; also cleaves des-Arg, bradykinin and neurotensin. | Inhibition of angiotensin can be determined using assays known in the art. For example, in vitro using a proliferation assay with rat cardiac fibroblasts as described in Naunyn Schmiedebergs Arch Pharmacol 1999 May;359(5):394-9. | Treatment for elevated angiotensin II and/or aldosterone levels, which can lead to vasoconstriction, impaired cardiac output and/or hypertension; Cardiovascular Disease; Cardiac Failure; Diabetes; Type II Diabetes; Proteinuria; Renal disorders, congestive heart failure. | 1989, 2000, 2001, and 2002.  | See Table 2, SEQ ID NO:Z for particular construct. |
| TR1 (OCIF; Osteoclastogenesis inhibitory factor; | Inhibits osteoclastogenesis and bone resorption, and induces fibroblast  | Coculture Assay for Osteoclastogenesis, Bone resorption assay using fetal long-bone organ culture  | Osteoporosis; Paget's disease; osteopenia; osteolysis; osteomyelitis; osteonecrosis; periodontal bone loss; osteoarthritis; rheumatoid arthritis; osteopetrosis; periodontal,  | 2016, 2017, 2085, 2086, 2529, 2530, 2531, 2532, 2555, 2556, 2557, and 2558.  | See Table 2, SEQ ID NO:Z for particular construct. |

**Table 1**

| <b>Therapeutic Protein:X</b>  | <b>Biological Activity</b>   | <b>Exemplary Activity Assay</b>  | <b>Preferred Indication: Y</b>  | <b>Construct ID</b>                           | <b>Therapeutic Protein:Z</b>                       |
|---|--|--|---|---|--|
| osteoprotegerin, OPG; tumor necrosis factor receptor superfamily member 11B precursor;) | proliferation.   | system, dentine resorption assay, and fibroblast proliferation assays are each described in Kwon et al., FASEB J. 12: 845-854 (1998).  | lytic, or metastatic bone disease; osteoclast differentiation inhibition; bone disorders; bone healing and regeneration; organ calcification; vascular calcification. |   |  |
| Human chemokine Ckbeta-7  | Chemotactic for both activated (CD3+) T cells and nonactivated (CD14-) lymphocytes and (CD4+) and (CD8+) T lymphocytes and (CD45RA+) T cells | Chemokine activities can be determined using assays known in the art: Methods in Molecular Biology, 2000, vol. 138: Chemokine Protocols. Edited by: A.E.I. Proudfoot, T.N.C. Wells, and C.A. Power. © Humana | Cancer; Wound healing; Inflammatory disorders; Immunoregulatory disorders; Atherosclerosis; Parasitic Infection; Rheumatoid Arthritis; Asthma; Autoimmune disorders.  | 2101, 2240, 2241, 2245, 2246, 2247, and 2248. | See Table 2, SEQ ID NO:Z for particular construct. |



**Table 1**

| Therapeutic Protein:X  | Biological Activity  | Exemplary Activity Assay  | Preferred Indication: Y   | Construct ID                                  | Therapeutic Protein:Z                              |
|--|--|---|---|---|--|
| CKbeta4 (HGB AN46; HE9DR66)  | Attracts and activates microbicidal leukocytes; Attracts CCR6-expressing immature dendritic cells and memory/effector T cells; B-cell chemotaxis; inhibits proliferation of myeloid progenitors; chemotaxis of PBMC's. | Press Inc., Totowa, NJ<br>Chemokine activities can be determined using assays known in the art: Methods in Molecular Biology, 2000, vol. 138: Chemokine Protocols. Edited by: A.E.I. Proudfoot, T.N.C. Wells, and C.A. Power. © Humana Press Inc., Totowa, NJ | Cancer; Solid Tumors; Chronic Infection; Autoimmune Disorders; Psoriasis; Asthma; Allergy; Hematopoiesis; Wound Healing; Bone Marrow Failure; Sclerosis; Sarcoidosis; Hyper-eosinophilic Syndrome; Lung Inflammation; Fibrotic Disorders; Atherosclerosis; Periodontal diseases; Viral diseases; Hepatitis.   | 2141, 2330, 2335, 2336, 2337, 2338, and 2348. | See Table 2, SEQ ID NO:Z for particular construct. |
| Leptin   | Controls obesity through regulation of appetite, reduction of body weight, and lowering of insulin and glucose level.  | in vivo modulation of food intake, reduction in body weight, and lowering of insulin and glucose levels in ob/ob mice, radioimmunoassay (RIA) and activation of the leptin receptor in a cell-based assay. Protein Expr Purif 1998 Dec;14(3):335-42           | Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); a Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X; Immunological Disorders; Immunosuppression. | 2146, 2184, 2186, and 2187.                   | See Table 2, SEQ ID NO:Z for particular construct. |
| IL-1 receptor antagonist (Anakinra; soluble interleukin-1 receptor; IRAP; KINERET; | Binds IL1 receptor without activating the target cells; inhibits the binding of IL1-alpha and IL1-beta; and neutralizes the biologic activity of IL1-alpha   | 1) Competition for IL-1 binding to IL-1 receptors in YT-NCI or C3H/HeJ cells (Carter et al., Nature 344: 633-638, 1990);<br>2) Inhibition of IL-1-  | Autoimmune Disease; Arthritis; Rheumatoid Arthritis; Asthma; Diabetes; Diabetes Mellitus; GVHD; Inflammatory Bowel Disorders; Chron's Disease; Ocular Inflammation; Psoriasis; Septic Shock; Transplant Rejection; Inflammatory Disorders; Rheumatic Disorders; Osteoporosis; Postmenopausal Osteoporosis;  | 2181, 2182, 2183, and 2185.                   | See Table 2, SEQ ID NO:Z for particular construct. |

**Table 1**

| Therapeutic Protein:X | Biological Activity                   | Exemplary Activity Assay  | Preferred Indication: Y   | Construct ID   | Therapeutic Protein:Z    |
|-----------------------|---------------------------------------|---|---|----------------|--------------------------|
| ANTRIL)               | and IL1-beta.                         | induced endothelial cell-leukocyte adhesion (Carter et al., Nature 344: 633-638, 1990);<br><br>3) Proliferation assays on A375-C6 cells, a human melanoma cell line highly susceptible to the antiproliferative action of IL-1 (Murai T et al., J. Biol. Chem. 276: 6797-6806, 2001). | Stroke.   |                |                          |
| TREM-1 (Triggering    | Mediates activation of neutrophil and | Secretion of cytokines, chemokines, degranulation,  | Inflammation; Sepsis; bacterial infection; autoimmune diseases; GVHD. | 2226 and 2230. | See Table 2, SEQ ID NO:Z |

**Table 1**

| Therapeutic Protein:X              | Biological Activity   | Exemplary Activity Assay   | Preferred Indication: Y | Construct ID | Therapeutic Protein:Z     |
|------------------------------------|---|--|-------------------------|--------------|---------------------------|
| Receptor Expressed on Monocytes 1) | monocytes; Stimulates neutrophil and monocyte-mediated inflammatory response; Promotes secretion of TNF, IL-8, and MCP-1; Induces neutrophil degranulation, Ca <sup>2+</sup> mobilization and tyrosine phosphorylation of extracellular signal-related kinase 1 (ERK1), ERK2 and phospholipase C- | and cell surface activation markers can be determined using assays described in Bouchon et al, J Immunol 2000 May 15;164(10):4991-5. |                         |              | for particular construct. |

**Table 1**

| Therapeutic Protein:X | Biological Activity   | Exemplary Activity Assay   | Preferred Indication: Y  | Construct ID   | Therapeutic Protein:Z                              |
|-----------------------|---|--|--|----------------|--|
| HCNCA73               | <p>gamma.</p> <p>Induces T-cell activation- expression of CD152 marker;</p> <p>Stimulates release of TNF-<math>\alpha</math> and MIP-1<math>\alpha</math> from immature, monocyte-derived dendritic cells;</p> <p>Promotes maturation of dendritic cells.</p> | <p>FMAT can be used to measure T-cell surface markers (CD69, CD152, CD71, HLA-DR) and T-cell cytokine production (e.g., IFN<math>\gamma</math> production). J. of Biomol. Screen. 4:193-204 (1999). Other T-cell proliferation assays:</p> <p>*Lymphocytes: a practical approach" edited by: SL Rowland, AJ McMichael - Chapter 6, pages 138-160 Oxford University Press</p> | <p>Autoimmune disorders; Inflammation of the gastrointestinal tract; Cancer; Colon Cancer; Allergy; Crohn's disease.</p> | 2244 and 2365. | See Table 2, SEQ ID NO:Z for particular construct. |

**Table 1**

| Therapeutic Protein:X  | Biological Activity  | Exemplary Activity Assay  | Preferred Indication: Y   | Construct ID  | Therapeutic Protein:Z                              |
|--|--|---|---|---|--|
|  |  | (2000); WO 01/21658 Examples 11-14, 16-17 and 33.   |   |   |  |
| VEGF-2 (Vascular Endothelial Growth Factor-2; VEGF-C)  | Promotes endothelial cell proliferation.   | VEGF activity can be determined using assays known in the art, such as those disclosed in International Publication No. WO0045835, for example.                         | Coronary artery disease; Critical limb ischemia; Vascular disease; proliferation of endothelial cells, both vascular and lymphatic. Antagonists may be useful as anti-angiogenic agents; Cancer.  | 2251, 2252, 2256, and 2257.   | See Table 2, SEQ ID NO:Z for particular construct. |
| HCHNF25 (jumping translocation breakpoint)   | Activates MIP1a Release in Dendritic Cells.  | Dendritic cell assays are well known in the art. For example, J. Immunol. 158:2919-2925 (1997); J. Leukoc. Biol. 65:822-828 (1999).                                     | Immune disorders; cancer.   | 2271, 2280, and 2320.   | See Table 2, SEQ ID NO:Z for particular construct. |
| HLDOU18 (Bone Morphogenic Protein 9 (BMP9); Growth differentiation factor-2 precursor (GDF-2 precursor)) | Activates L6/GSK3 kinase assay.  | Assays for activation of GSK3 kinase activity are well known in the art. For example, Biol. Chem. 379(8-9): (1998) 1101-1110.; Biochem J. 1993 Nov 15;296 ( Pt 1):15-9. | Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X. | 2328, 2340, 2350, 2351, 2359, 2362, 2367, 2369, 2370, 2473, 2623, 2624, 2625, 2631, 2632, 2633. | See Table 2, SEQ ID NO:Z for particular construct. |
| Glucagon-Like Peptide 1 (GLP1; Insulinotropin)   | Stimulates the synthesis and release of insulin; enhances the sensitivity of [H]-glucose uptake assay. (J adipose, muscle, and liver | GLP1 activity may be assayed in vitro using a [3-   | Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin   | 2448, 2455, 2456, 2457, 2803, 2804, 2900, 2904, 2945, 2964, 2982, 3070, 2802, 3027, 3028, 3045, | See Table 2, SEQ ID NO:Z for particular construct. |

**Table 1**

| Therapeutic Protein:X | Biological Activity   | Exemplary Activity Assay  | Preferred Indication: Y   | Construct ID   | Therapeutic Protein:Z                              |
|-----------------------|---|---|---|--|--|
|                       | tissues towards insulin; stimulates glucose uptake; slows the digestive process; suppresses appetite; blocks the secretion of glucagon.                     | 274(43):30864-30873).   | dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X. | 3046, 3069, 3071, 3072, 3085, 3086, 3087, 3140, 3309 |  |
| Exendin-4 (AC-2993)   | Stimulates the synthesis and release of insulin; enhances the sensitivity of adipose, muscle, and liver tissues towards insulin; stimulates glucose uptake; | Exendin-4 activity may be assayed in vitro using a [ <sup>3</sup> -H]-glucose uptake assay. (J Biol Chem 1999 Oct 22; 274(43):30864-30873). | Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A  | 2469 and 2470.                                       | See Table 2, SEQ ID NO:Z for particular construct. |

**Table 1**

| Therapeutic Protein:X   | Biological Activity  | Exemplary Activity Assay   | Preferred Indication: Y  | Construct ID                  | Therapeutic Protein:Z                              |
|---|--|--|--|-------------------------------|--|
|   | slows the digestive process; suppresses appetite; blocks the secretion of glucagon.  |  | Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X.   |                               |  |
| T20 (T20 HIV inhibitory peptide, DP178; DP178 HIV inhibitory peptide)   | a peptide from residues 643-678 of the HIV gp41 transmembrane protein ectodomain which binds to gp41 in its resting state and prevents transformation to the fusogenic state   | Virus inhibition assays as described in Zhang et al., Sept. 26 2002, Sciencexpress (www.sciencexpress.org).  | HIV; AIDS; SIV (simian immunodeficiency virus) infection.  | 7777, 2672, 2673              | See Table 2, SEQ ID NO:Z for particular construct. |
| T1249 (T1249 HIV inhibitory peptide; T1249 anti-HIV peptide)  | a second generation HIV fusion inhibitor   | Virus inhibition assays as described in Zhang et al., Sept. 26 2002, Sciencexpress (www.sciencexpress.org).  | HIV; AIDS; SIV (simian immunodeficiency virus) infection   | 9999, 2667, 2670, 2946        | See Table 2, SEQ ID NO:Z for particular construct. |
| Interferon Hybrids, specifically preferred: IFNalpha A/D hybrid (BgIII version) IFNalpha A/D hybrid (PvuII version) IFNalpha A/F hybrid | Confers a range of cellular responses including antiviral, antiproliferative, antitumor and immunomodulatory activities; stimulate production of two enzymes: a protein kinase and an oligoadenylate synthetase. Also, modulates MHC antigen expression, NK cell | Anti-viral assay: Rubinstein S, Familletti PC, Pestka S. (1981) Convenient assay for interferons. J. Virol. 37(2):755-8; Anti-proliferation assay: Gao Y, et al (1999) Sensitivity of an epstein-barr virus-positive tumor line, Daudi, to alpha interferon correlates with expression of a GC-rich viral transcript. Mol Cell Biol. 19(11):7305-13. | Viral infections; HIV Infections; Hepatitis; Chronic Hepatitis; Hepatitis B; Chronic Hepatitis D; B; Hepatitis C; Chronic Hepatitis C; Hepatitis D; Chronic Hepatitis D; Human Papillomavirus; Herpes Simplex Virus Infection; External Condylomata Acuminata; HIV; HIV Infection; Oncology; Cancer; Solid Tumors; Melanoma; Malignant Melanoma; Renal Cancer (e.g., Renal Cell Carcinoma); Lung Cancer (e.g., Non-Small Cell Lung Cancer or Small Cell Lung Cancer) Colon Cancer; Breast Cancer; Liver Cancer; Prostate Cancer; Bladder Cancer; Gastric Cancer; Sarcoma; AIDS-Related Kaposi's Sarcoma; | 2875, 2872, 2876, 2874, 2873. | See Table 2, SEQ ID NO:Z for particular construct. |

**Table 1**

| Therapeutic Protein:X   | Biological Activity  | Exemplary Activity Assay | Preferred Indication: Y   | Construct ID | Therapeutic Protein:Z |
|---|--|--------------------------|---|--------------|-----------------------|
| IFNalpha A/B hybrid<br>IFNbeta 1/alpha D hybrid<br>(IFNbeta-1/alpha-1 hybrid)<br>IFNalpha/beta hybrid | activity and IFNg production and IL12 production in monocytes. |                          | Lymphoma; T Cell Lymphoma; Cutaneous T-Cell Lymphoma; Non-Hodgkin's Lymphoma; Brain Cancer; Glioma; Glioblastoma Multiforme; Cervical Dysplasia; Leukemia; Preleukemia; Bone Marrow Disorders; Bone Disorders; Hairy Cell Leukemia; Chronic Myelogenous Leukemia; Hematological Malignancies; Hematological Disorders; Multiple Myeloma; Bacterial Infections; Chemoprotection; Thrombocytopenia; Multiple Sclerosis; Pulmonary Fibrosis; Age-Related Macular Degeneration; Macular Degeneration; Crohn's Disease; Neurological Disorders; Arthritis; Rheumatoid Arthritis; Ulcerative Colitis; Osteoporosis; Osteopenia; Osteoclastogenesis; Fibromyalgia; Sjogren's |              |                       |



**Table 1**

| Therapeutic Protein:X                                       | Biological Activity   | Exemplary Activity Assay   | Preferred Indication: Y   | Construct ID | Therapeutic Protein:Z                              |
|---|---|--|---|--------------|--|
|   |   |  | Syndrome; Chronic Fatigue Syndrome; Fever; Hemorrhagic Fever; Viral Hemorrhagic Fevers; Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X. |              |  |
| B-type natriuretic peptide (BNP, brain natriuretic peptide) | stimulates smooth muscle relaxation and vasodilation, natriuresis, and suppression of renin-angiotensin and endothelin. | Inhibition of angiotensin can be determined using assays known in the art, for example using an in vitro proliferation assay with rat cardiac fibroblasts as described in Naunyn Schmiedebergs Arch Pharmacol 1999 May;359(5):394-9. Vasodilation can be measured in animals by measuring the myogenic responses of small renal arteries in an isobaric arteriograph system ( <i>see</i> Am J Physiol Regul Integr Comp Physiol 2002 | Congestive heart failure; cardiac volume overload; cardiac decompensation; Cardiac Failure; Left Ventricular Dysfunction; Dyspnea   | 3119, 8888.  | See Table 2, SEQ ID NO:Z for particular construct. |

**Table 1**

| <b>Therapeutic Protein:X</b>  | <b>Biological Activity</b>  | <b>Exemplary Activity Assay</b>  | <b>Preferred Indication: Y</b>  | <b>Construct ID</b> | <b>Therapeutic Protein:Z</b>                       |
|---|---|--|---|---------------------|--|
|   |   | Aug:283(2):R349-R355).<br>Natriuresis is determined by measuring the amount of sodium in the urine.  |   |                     |  |
| $\alpha$ -defensin, including alpha 1 defensin, alpha 2 defensin, alpha 3 defensin (myeloid-related defensin; DEFA1; neutrophil-specific defensin; CAF) | Suppression of HIV replication; active against bacteria, fungi, and enveloped viruses.                                    | Virus inhibition assays as described in Zhang et al., Sept. 26 2002, Sciencepress (www.sciencepress.org).  | HIV, AIDS; ARC.   | 3208, 3209, 3210.   | See Table 2, SEQ ID NO:Z for particular construct. |
| Phosphatonin (matrix extracellular phosphoglycoprotein, MEPE)   | Regulation of phosphate metabolism.   | Blood phosphate levels can be measured using methods known in the art such as the Hypophosphatemic Rat Bioassay. Zoolog Sci 1995 Oct;12(5):607-10. | Hyperphosphatemia; Hyperphosphatemia in chronic renal failure; hypophosphatemia; Osteomalacia; Rickets; X-linked dominant hypophosphatemic rickets/osteomalacia (XLH); autosomal dominant hypophosphatemic rickets/osteomalacia (ADHR); tumor-induced rickets/osteomalacia (TIO). | 3238.               | See Table 2, SEQ ID NO:Z for particular construct. |
| P1pal-12 (pepducin, PAR1-based pepducin)  | Regulation of protease-activated receptor (PAR) signal transduction and thrombin-mediated aggregation of human platelets. | Platelet aggregation can be measured using methods known in the art such as described in Nature Medicine 2002 Oct; 8(10): 1161-1165.               | Protection against systemic platelet activation, thrombus, heart attack, stroke, and/or coagulation disorders.  | 3274.               | See Table 2, SEQ ID NO:Z for particular construct. |
| P4pal-10 (pepducin, PAR4-based pepducin)  | Regulation of protease-activated receptor (PAR) signal transduction and thrombin-mediated aggregation of human            | Platelet aggregation can be measured using methods known in the art such as described in Nature Medicine 2002 Oct; 8(10):                          | Protection against systemic platelet activation, thrombus, heart attack, stroke, and/or coagulation disorders.  | 3275.               | See Table 2, SEQ ID NO:Z for particular construct. |

**Table 1**

| Therapeutic Protein:X | Biological Activity   | Exemplary Activity Assay  | Preferred Indication: Y  | Construct ID | Therapeutic Protein:Z                              |
|-----------------------|---|---|--|--------------|--|
| HRDFD27               | platelets.<br>Involved in the proliferation of T cells; Production of TNFgamma. | 1161-1165.<br>T-cell proliferation can be measured using assays known in the art. For example, "Lymphocytes: a practical approach" edited by: SL Rowland, AJ McMichael – chapter 6, pages 138-160 Oxford University Press (2000); and "Current Protocols on CD-ROM" section 3.12 Proliferation Assays for T-cell Function John Wiley & Sons, Inc. (1999). | Chemoprotection; Adjunct to Chemotherapy; Inflammatory disorders; Cancer; Leukemia; Myelocytic leukemia; Neutropenia, Primary neutropenias (e.g.; Kostmann syndrome); Secondary neutropenia; Prevention of neutropenia; Prevention and treatment of neutropenia in HIV-infected patients; Prevention and treatment of neutropenia associated with chemotherapy; Infections associated with neutropenias; Myelopysplasia; Autoimmune disorders; Psoriasis; Mobilization of hematopoietic progenitor cells; Wound Healing; Autoimmune Disease; Transplants; Bone marrow transplants; Acute myelogenous leukemia; | 2361         | See Table 2, SEQ ID NO:Z for particular construct. |

**Table 1**

| Therapeutic Protein:X   | Biological Activity   | Exemplary Activity Assay  | Preferred Indication: Y  | Construct ID | Therapeutic Protein:Z                              |
|---|---|---|--|--------------|--|
|   |   |   | Lymphoma, Non-Hodgkin's lymphoma; Acute lymphoblastic leukemia; Hodgkin's disease; Accelerated myeloid recovery; Glycogen storage disease  |              |  |
| HWHGZ51 (CD59; Metastasis-associated GPI-adhered protein homolog) | Stimulates an immune response and induces inflammation by inducing mononuclear cell, eosinophil and PMN infiltration; Inhibits growth of breast cancer, ovarian cancer, leukemia, and melanoma; Overexpressed in colon, lung, breast and rectal | The ability to affect chondrocyte differentiation can be measured using methods known in the art, such as described in Bone (1995) Sep; 17(3):279-86. | Skeletal diseases and disorders; Musculoskeletal diseases and disorders; Bone fractures and/or breaks; Osteoporosis (postmenopausal, senile, or idiopathic juvenile); Gout and/or pseudogout; Paget's disease; Osteoarthritis; Tumors and/or cancers of the bone (osteochondromas, benign chondromas, chondroblastomas, chondromyxoid fibromas, osteoid osteomas, giant cell tumors, multiple myelomas, osteosarcomas, fibrosarcomas, malignant fibrous histiocytomas, chondrosarcomas, Ewing's tumors, and/or | 2407, 2408   | See Table 2, SEQ ID NO:Z for particular construct. |

**Table 1**

| Therapeutic Protein:X | Biological Activity  | Exemplary Activity Assay | Preferred Indication: Y   | Construct ID | Therapeutic Protein:Z |
|-----------------------|--|--------------------------|---|--------------|-----------------------|
|                       | tumors; Regulates glucose and/or FFA uptake by adipocytes and skeletal muscle; Induces redifferentiation of chondrocytes |                          | malignant lymphomas); Bone and joint infections (osteomyelitis and/or infectious arthritis); Charcot's joints; Heel spurs; Sever's disease; Sport's injuries; Cancer; Solid Tumors; Melanoma; Malignant Melanoma; Renal Cancer (e.g., Renal Cell Carcinoma); Lung Cancer (e.g., Non-Small Cell Lung Cancer or Small Cell Lung Cancer) Colon Cancer; Breast Cancer; Liver Cancer; Prostate Cancer; Bladder Cancer; Gastric Cancer; Sarcoma; AIDS-Related Kaposi's Sarcoma; Lymphoma; T Cell Lymphoma; Cutaneous T-Cell Lymphoma; Non-Hodgkin's Lymphoma; Brain Cancer; Glioma; Glioblastoma Multiforme; Cervical Dysplasia; Leukemia; Preleukemia; Bone Marrow Disorders; Bone |              |                       |

**Table 1**

| Therapeutic Protein:X | Biological Activity | Exemplary Activity Assay | Preferred Indication: Y   | Construct ID | Therapeutic Protein:Z |
|-----------------------|---------------------|--------------------------|---|--------------|-----------------------|
|                       |                     |                          | Disorders; Hairy Cell Leukemia; Chronic Myelogenous Leukemia; Hematological Malignancies; Hematological Disorders; Multiple Myeloma; Kidney diseases and disorders; Shonlein-Henoch purpura, Berger disease, celiac disease, dermatitis herpetiformis, Chron disease; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or |              |                       |

**Table 1**

| Therapeutic Protein:X           | Biological Activity   | Exemplary Activity Assay  | Preferred Indication: Y  | Construct ID | Therapeutic Protein:Z                              |
|---------------------------------|---|---|--|--------------|--|
| C17 (cytokine-like protein C17) | Inhibits glucose and/or FFA uptake by adipocytes; Induces proliferation of kidney mesangial cells; Regulation of cytokine production and antigen presentation | Proliferation of kidney mesangial cells can be assayed using techniques described in J. Investig. Med. (1998) Aug; 46(6):297-302. | Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X; Kidney disorders; Hyperinsulinemia; Hypoinsulinemia; Immunological disorders (e.g. arthritis, asthma, immunodeficiency diseases, AIDS, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, T-cell mediated cytotoxicity, host-versus-graft disease, autoimmunity disorders, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjorgren's disease, scleroderma) | 2489, 2490   | See Table 2, SEQ ID NO:Z for particular construct. |

**Table 1**

| Therapeutic Protein:X | Biological Activity                                  | Exemplary Activity Assay                           | Preferred Indication: Y  | Construct ID | Therapeutic Protein:Z    |
|-----------------------|--|--|--|--------------|--------------------------|
|                       |  |  | diseases and disorders; Developmental diseases and disorders; Hepatic diseases and disorders; Cancer (particularly leukemia); Immunological disorders (e.g. arthritis, asthma, immunodeficiency diseases, AIDS, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, T-cell mediated cytotoxicity, host-versus-graft disease, autoimmunity disorders, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjorgren's disease, scleroderma) |              |                          |
| HDPBQ71               | Regulates production and secretion of IFN $\gamma$ ; | Such assays that may be used or routinely modified | Blood disorders and infection (e.g., viral infections, tuberculosis, infections associated   | 2515, 2545   | See Table 2, SEQ ID NO:Z |



**Table 1**

| Therapeutic Protein:X | Biological Activity                                    | Exemplary Activity Assay   | Preferred Indication: Y   | Construct ID | Therapeutic Protein:Z     |
|-----------------------|--|--|---|--------------|---------------------------|
|                       | Activation of myeloid cells and/or hematopoietic cells | to test immunomodulatory activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include the assays disclosed in Miraglia et al., J Biomolecular Screening 4:193-204 (1999); Rowland et al., ""Lymphocytes: a practical approach"" Chapter 6:138-160 (2000); Gonzalez et al., J Clin Lab Anal 8(5):225-233 (1995); Billiau et al., Ann NY Acad | with chronic granulomatous disease and malignant osteoporosis); Autoimmune disease (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis); Immunodeficiency, boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response; Inflammation and inflammatory disorders; Idiopathic pulmonary fibrosis; Neoplastic diseases (e.g., leukemia, lymphoma, melanoma); Neoplasms and cancers, such as, for example, leukemia, lymphoma, melanoma, and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer; Benign dysproliferative disorders and pre-neoplastic conditions, such as, for |              | for particular construct. |

**Table 1**

| Therapeutic Protein:X         | Biological Activity                                | Exemplary Activity Assay  | Preferred Indication: Y  | Construct ID | Therapeutic Protein:Z    |
|-------------------------------|--|---|--|--------------|--------------------------|
|                               |  | Sci 856:22-32 (1998); Boehm et al., Annu Rev Immunol 15:749-795 (1997), and Rheumatology (Oxford) 38(3):214-20 (1999) | example, hyperplasia, metaplasia, and/or dysplasia; Anemia; Pancytopenia; Leukopenia; Thrombocytopenia; Hodgkin's disease; Acute lymphocytic anemia (ALL); Plasmacytomas; Multiple myeloma; Burkitt's lymphoma; Arthritis; AIDS; Granulomatous disease; Inflammatory bowel disease; Sepsis; Neutropenia; Neutrophilia; Psoriasis; Suppression of immune reactions to transplanted organs and tissues; Hemophilia; Hypercoagulation; Diabetes mellitus; Endocarditis; Meningitis; Lyme Disease; Asthma; Allergy |              |                          |
| Oscar (osteoclast-associated) | Regulator of osteoclast differentiation; regulator | Assay to detect osteoclast differentiation is described   | Skeletal diseases and disorders; Musculoskeletal diseases and disorders; Bone fractures and/or   | 2571, 2749   | See Table 2, SEQ ID NO:Z |

**Table 1**

| Therapeutic Protein:X            | Biological Activity                           | Exemplary Activity Assay                                  | Preferred Indication: Y  | Construct ID                                   | Therapeutic Protein:Z     |
|----------------------------------|---|---|--|--|---------------------------|
| receptor isoform-3)              | of innate and adaptive immune responses       | in J. Exp. Med. (2002) Jan 21; 195(2):201-9.              | breaks; Osteoporosis (postmenopausal, senile, or idiopathic juvenile); Gout and/or pseudogout; Paget's disease; Osteoarthritis; Tumors and/or cancers of the bone (osteochondromas, benign chondromas, chondroblastomas, chondromyxoid fibromas, osteoid osteomas, giant cell tumors, multiple myelomas, osteosarcomas, fibrosarcomas, malignant fibrous histiocytomas, chondrosarcomas, Ewing's tumors, and/or malignant lymphomas); Bone and joint infections (osteomyelitis and/or infectious arthritis); Charcot's joints; Heel spurs; Sever's disease; Sport's injuries |  | for particular construct. |
| Tumstatin (T5, T7 or T8 peptide; | Inhibits angiogenesis; Inhibits tumor growth; | A tumor cell proliferation assay is described in J. Biol. | Cancer; Solid Tumors; Melanoma; Malignant Melanoma; Renal Cancer (e.g., Renal Cell   | 2647, 2648, 2649, 2650, 2943, 2944, 3047, 3048 | See Table 2, SEQ ID NO:Z  |

**Table 1**

| Therapeutic Protein:X      | Biological Activity                | Exemplary Activity Assay  | Preferred Indication: Y  | Construct ID           | Therapeutic Protein:Z     |
|----------------------------|------------------------------------|---|--|------------------------|---------------------------|
| $\alpha 3(IV)(NC1)$        | Inhibits protein synthesis         | Chem. (1997) 272:20395-20401.<br>Protein synthesis can be measured as described in Science (2002) Jan 4; 295(5552):140-3. | Carcinoma); Lung Cancer (e.g., Non-Small Cell Lung Cancer or Small Cell Lung Cancer) Colon Cancer; Breast Cancer; Liver Cancer; Prostate Cancer; Bladder Cancer; Gastric Cancer; Sarcoma; AIDS-Related Kaposi's Sarcoma; Lymphoma; T Cell Lymphoma; Cutaneous T-Cell Lymphoma; Non-Hodgkin's Lymphoma; Brain Cancer; Glioma; Glioblastoma Multiforme; Cervical Dysplasia; Leukemia; Preleukemia; Bone Marrow Disorders; Bone Disorders; Hairy Cell Leukemia; Chronic Myelogenous Leukemia; Hematological Malignancies; Hematological Disorders; Multiple Myeloma; Angiogenesis |                        | for particular construct. |
| CNTF (Ciliary neurotrophic | Enhances myelin formation; Reduces | Regulation of myelin formation can be assayed as  | Neurological and neural diseases and disorders, particularly diseases and disorders associated   | 2724, 2725, 3171, 3172 | See Table 2, SEQ ID NO:Z  |

**Table 1**

| Therapeutic Protein:X  | Biological Activity  | Exemplary Activity Assay   | Preferred Indication: Y   | Construct ID                 | Therapeutic Protein:Z                              |
|--|--|--|---|------------------------------|--|
| factor)  | photoreceptor degredation; Regulates calcium currents  | described in J. Neurosci. (2002) Nov. 1; 22(21):9221-7.  | with myelin and demyelination, such as, for example, ALS, multiple sclerosis, Huntington's disease; Neuronal and spinal cord injuries; Disorders of the eye, such as, for example, retinitis pigmentosa, blindness, color-blindness, macular degeneration.  |                              | for particular construct.                          |
| Somatostatin (Octreotide; octreotide acetate; Sandostating LAR®) | Inhibits growth hormone, glucagons and insulin; Suppresses LF response to GnRH; Decreases splanchnic blood flow; Inhibits release of serotonin, gastrin, vasoactive intestinal peptide, secretin, motilin, | Inhibition of growth hormone release in humans by somatostatin can be measured as described in J. Clin. Endocrinol. Metab. (1973) Oct; 37(4):632-4. Inhibition of insulin secretion by somatostatin can be measured as | Cancer; Metastatic carcinoid tumors; Vasoactive Intestinal Peptide secreting adenomas; Diarrhea and Flushing; Prostatic disorders and cancers; Breast cancer; Gastrointestinal disorders and cancers; Cancers of the endocrine system; Head and neck paragangliomas; Liver disorders and cancers; Nasopharyngeal cancers; Thyroid disorders and cancers; Acromegaly; Carcinoid Syndrome; Gallbladder disorders, such as | 2798, 2825, 2830, 2831, 2902 | See Table 2, SEQ ID NO:Z for particular construct. |

**Table 1**

| Therapeutic Protein:X | Biological Activity         | Exemplary Activity Assay                                  | Preferred Indication: Y  | Construct ID | Therapeutic Protein:Z |
|-----------------------|-----------------------------|---|--|--------------|-----------------------|
|                       | and pancreatic polypeptide. | described in the Lancet (1973) Dec. 8; 2(7841):1299-1301. | gallbladder contractility diseases and abnormal bile secretion; Psoriasis; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X; Kidney disorders; Neurological disorders and diseases, including |              |                       |

**Table 1**

| Therapeutic Protein:X                     | Biological Activity  | Exemplary Activity Assay   | Preferred Indication: Y  | Construct ID | Therapeutic Protein:Z                              |
|---|--|--|--|--------------|--|
|   |  |  | Alzheimers Disease, Parkinson's disease and dementia; Neuropsychotic disorders, including Bipolar affective disorder; Rheumatoid arthritis; Hypertension; Intracranial hypertension; Esophageal varices; Graves' disease; Seizures; Epilepsy; Gastritis; Angiogenesis;   |              |  |
| IL-22 (IL22, interleukin-22; IL17D, IL27) | Stimulates glucose uptake in skeletal muscle cells; increases skeletal muscle insulin sensitivity. | IL-22 activity may be assayed in vitro using a [3-H]-glucose uptake assay. (J Biol Chem 1999 Oct 22; 274(43):30864-30873). | Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin-dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or | 2901, 2903   | See Table 2, SEQ ID NO:Z for particular construct. |

**Table 1**

| Therapeutic Protein:X | Biological Activity  | Exemplary Activity Assay  | Preferred Indication: Y   | Construct ID                 | Therapeutic Protein:Z                              |
|-----------------------|--|---|---|------------------------------|--|
|                       |  |   | Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X.  |                              |  |
| HCE1P80               | Stimulates glucose uptake in; increases insulin sensitivity. | HCE1P80 activity may be assayed in vitro using a [ <sup>3</sup> -H]-glucose uptake assay. (J Biol Chem 1999 Oct 22; 274(43):30864-30873). | Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of | 2908, 3049, 3050, 3051, 3052 | See Table 2, SEQ ID NO:Z for particular construct. |



**Table 1**

| Therapeutic Protein:X   | Biological Activity                                       | Exemplary Activity Assay   | Preferred Indication: Y   | Construct ID     | Therapeutic Protein:Z                              |
|---|---|--|---|------------------|--|
|   |   |  | Body Weight; Suppression of Appetite; Syndrome X.   |                  |  |
| HDRMI82   | Stimulates glucose uptake; increases insulin sensitivity. | HDRMI82 activity may be assayed in vitro using a [3-H]-glucose uptake assay. (J Biol Chem 1999 Oct 22; 274(43):30864-30873). | Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin-dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X.   | 2909             | See Table 2, SEQ ID NO:Z for particular construct. |
| HDALV07 (adiponectin; gelatin-binding 28k protein precursor; adipose most abundant gene transcript; APM-1; GBP28; ACRP30; ADIPOQ) | Modulates insulin action                                  | Insulin activity may be assayed in vitro using a [3-H]-glucose uptake assay. (J Biol Chem 1999 Oct 22; 274(43):30864-30873). | Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X; Hyperglycemia; Familial combined hyperlipidemia; Metabolic syndrome; Inflammatory disorders; Atherogenic disorders | 3053, 3055, 3056 | See Table 2, SEQ ID NO:Z for particular construct. |
| C Peptide   | An insulin precursor involved in insulin regulation       | C-peptide concentrations can be measured using assays well known in the art,   | Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia;  | 3088, 3149       | See Table 2, SEQ ID NO:Z for particular            |

**Table 1**

| Therapeutic Protein:X                             | Biological Activity   | Exemplary Activity Assay  | Preferred Indication: Y   | Construct ID | Therapeutic Protein:Z                              |
|---|---|---|---|--------------|--|
|   |   | such as the one described in PNAS (1970) Sep; 67(1):148-55  | Hyperketonemia; Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X; Hyperglycemia; Familial combined hyperlipidemia; Metabolic syndrome |              | construct.   |
| HCBOG68 (enteric adipokine; Fat SID; proline rich | Controls proliferation/ differentiation or metabolism/ physiology/pathology/ of | Activation of cAMP-mediated transcription in adipocytes can be assayed using methods known in the | Treatment of Obesity; treatment of Diabetes; suppression of body weight gain; suppression of appetite. Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes;   | 3106, 3270   | See Table 2, SEQ ID NO:Z for particular construct. |

**Table 1**

| Therapeutic Protein:X  | Biological Activity  | Exemplary Activity Assay  | Preferred Indication: Y   | Construct ID                        | Therapeutic Protein:Z                              |
|--|--|---|---|-------------------------------------|--|
| acidic protein)  | adipocytes and adipose tissue in response to dietary conditions. | art (Berger et al., Gene 66:1-10 (1998); Cullen and Malm, Methods in Enzymol 216:362-368 (1992); Henthorn et al., Proc Natl Acad Sci USA 85:6342-6346 (1988); Reusch et al., Mol Cell Biol 20(3):1008-1020 (2000); and Klemm et al., J Biol Chem 273:917-923 (1998)). | Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X.<br>Other indications for antibodies and/or antagonists, include treatment of weight loss; treatment of AIDS wasting; appetite stimulant; treatment of cachexia.  |                                     |  |
| PYY (Peptide YY), including PYY <sub>3-36</sub> (amino acid residues 31-64 of full length PYY, amino acid residues 3-36 of mature PYY) | Decreases appetite; increases satiety; decreases food intake.    | Appetite and food intake can be measured by methods known in the art (Batterham et al. Nature 2002; 418:650654)   | Most preferred: Treatment of Obesity; treatment of Diabetes; suppression of body weight gain; suppression of appetite.<br>Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X.<br>Other indications for antibodies, antagonists: treatment of weight loss; treatment of AIDS | 3108, 3109, 3281, 3117, 3118, 3282. | See Table 2, SEQ ID NO:Z for particular construct. |

**Table 1**

| Therapeutic Protein:X | Biological Activity            | Exemplary Activity Assay  | Preferred Indication: Y  | Construct ID | Therapeutic Protein:Z                              |
|-----------------------|--------------------------------|---|--|--------------|--|
| WNT10b                | Inhibits adipogenesis.         | WNT10b activity can be measured using adipogenesis inhibition assays (Ross et al., Science 2000; 289(5481):950-953                              | wasting; appetite stimulant; treatment of cachexia.<br><br>Most preferred: Treatment of Obesity; suppression of body weight gain; suppression of appetite.<br>Other indications: Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM).  | 3141         | See Table 2, SEQ ID NO:Z for particular construct. |
| WNT11                 | Promotes cardiogenesis.        | WNT11 activity can be measured using assays known in the art, including cardiogenesis assays (Eisenberg et al., Dev Dyn 1999 Sep;216(1):45-58). | Treatment of Cardiovascular disorders; Congestive Heart Failure; Myocardial Infarction.  | 3142         | See Table 2, SEQ ID NO:Z for particular construct. |
| Herstatin             | Inhibits cancer proliferation. | Herstatin activity can be measured using cell proliferation assays known in the art (Doherty et al., PNAS 1999; 96(19):10869-10874.             | Oncology; Cancer; Solid Tumors; Melanoma; Malignant Melanoma; Renal Cancer (e.g., Renal Cell Carcinoma); Lung Cancer (e.g., Non-Small Cell Lung Cancer or Small Cell Lung Cancer); Colon Cancer; Breast Cancer; Liver Cancer; Prostate Cancer; Bladder Cancer; Gastric Cancer; Sarcoma; AIDS-Related Kaposi's Sarcoma; Lymphoma; T Cell Lymphoma; Cutaneous T-Cell Lymphoma; Non-Hodgkin's Lymphoma; Brain Cancer; Glioma; Glioblastoma Multiforme; Cervical Dysplasia; Leukemia; Preleukemia; Hairy Cell Leukemia; Chronic Myelogenous Leukemia; Hematological Malignancies; Hematological Disorders; Multiple Myeloma. | 3143         | See Table 2, SEQ ID NO:Z for particular construct. |
| Adrenomedullin        | stimulates vasodilation;       | Vasodilation can be   | Treatment of Congestive Heart Failure;   | 3144         | See Table 2,                                       |

**Table 1**

| <b>Therapeutic Protein:X</b> | <b>Biological Activity</b>                    | <b>Exemplary Activity Assay</b>   | <b>Preferred Indication: Y</b>  | <b>Construct ID</b> | <b>Therapeutic Protein:Z</b>                       |
|------------------------------|---|---|---|---------------------|--|
|                              | promotes bone growth.                         | measured using assays known in the art (Ashton et al. Pharmacology 2000; 61(2):101-105. The promotion of bone growth can be measured using assays known in the art, such as the osteoblast proliferation assay (Cornish et al. Am J Physiol 1997 Dec;273(6 Pt 1):E1113-20). | Hypertension; Myocardial Infarction; Septic Shock; Osteoporosis; Postmenopausal osteoporosis; Osteopenia.   |                     | SEQ ID NO:Z for particular construct.              |
| Nogo Receptor                | Receptor for the axon growth inhibitor, Nogo. | The promotion of axon regeneration and growth can be measured using assays known in the art (Fournier et  | Treatment of Central Nervous System Damage; Spinal Cord Injury; Peripheral Nerve Damage; Neurodegenerative Diseases; Parkinson's Disease; Alzheimer's Disease; Huntington's | 3184, 3185          | See Table 2, SEQ ID NO:Z for particular construct. |

**Table 1**

| Therapeutic Protein:X                                | Biological Activity   | Exemplary Activity Assay   | Preferred Indication: Y   | Construct ID | Therapeutic Protein:Z                              |
|--|---|--|---|--------------|--|
|  |   | al. Nature 2001; 409(6818):341-346).   | Disease; Amyotrophic Lateral Sclerosis; Progressive Supranuclear Palsy; Creutzfeld-Jacob Disease; Motor Neuron Disease.   |              |  |
| CART (Cocaine- and Amphetamine-Regulated Transcript) | Inhibits food intake and fat storage; promotes lipid oxidation. | Appetite and food intake can be measured by methods known in the art (Batterham et al. Nature 2002; 418:650654)                                      | Most preferred: Treatment of Obesity; suppression of body weight gain; suppression of appetite.<br>Other indications: Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM).  | 3232         | See Table 2, SEQ ID NO:Z for particular construct. |
| RegIV (Colon Specific Gene; Colon Specific Protein)  | Stimulates glucose uptake; increases insulin sensitivity.       | RegIV activity may be assayed in vitro using a [3-H]-glucose uptake assay. (J Biol Chem 1999 Oct 22; 274(43):30864-30873).                           | Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X. | 2910.        | See Table 2, SEQ ID NO:Z for particular construct. |
| Cosyntropin (Cortrosyn) (CAS-16960-16-0)             | Synthetic corticotropin; stimulates the release of cortisol.    | The activity of cosyntropin can be assessed in vivo by measuring serum cortisol levels. (Frank et al. J. Am. Vet. Med. Assoc. 1998 212(10):1569-71). | Endocrine; Addison's disease; Cushing's syndrome; pituitary dysfunction; acute adrenal crisis   |              | SEQ ID: NO:2198                                    |

**Table 1**

| Therapeutic Protein:X                  | Biological Activity   | Exemplary Activity Assay   | Preferred Indication: Y  | Construct ID | Therapeutic Protein:Z |
|--|---|--|--|--------------|-----------------------|
| Pexiganan Acetate (CAS-172820-23-4)    | Disrupts bacterial membranes.   | Pexiganan acetate activity can be assessed using in vitro antibacterial assays known in the art. (Zasloff et al., Antimicrobial Agents and Chemotherapy 1999, 43:782-788). | Treatment of Infectious Diseases; Treatment of Bacterial Infections.   |              | SEQ ID NO: 2199       |
| Pramlintide (Amylin) (CAS-151126-32-8) | Slows gastric emptying; decreases food intake.  | Appetite and food intake can be measured by methods known in the art (Batterham et al. Nature 2002; 418:650654)  | Treatment of Obesity; treatment of Diabetes; suppression of body weight gain; suppression of appetite; treatment of endocrine disorders; Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X.<br>Other indications for antibodies, antagonists: treatment of weight loss; treatment of AIDS wasting; appetite stimulant; treatment of cachexia. |              | SEQ ID NO: 2200       |
| Teriparatide (CAS-52232-67-4)          | Acts in conjunction with calcitonin to control calcium and phosphate metabolism; elevates blood calcium level; stimulates the activity of | Adenyl cyclase stimulation in rat osteosarcoma cells, ovariectomized rat model of osteoporosis: IUBMB Life 2000 Feb;49(2):131-5  | Bone Disorders; Fracture prevention; Hypercalcemia; Malignant hypercalcemia; Osteoporosis; Paget's disease; Osteopenia, Osteoclastogenesis; osteolysis; osteomyelitis; osteonecrosis; periodontal bone loss; osteoarthritis; rheumatoid arthritis; osteopetrosis;  |              | SEQ ID NO: 2201       |

**Table 1**

| Therapeutic Protein:X  | Biological Activity   | Exemplary Activity Assay   | Preferred Indication: Y  | Construct ID | Therapeutic Protein:Z |
|--|---|--|--|--------------|-----------------------|
|  | osteocytes; enhances absorption of Ca <sup>++</sup> /Pi from small intestine into blood; promotes reabsorption of Ca <sup>++</sup> and inhibits Pi by kidney tubules. |  | periodontal, lytic, or metastatic bone disease; osteoclast differentiation inhibition; bone disorders; bone healing and regeneration.      |              |                       |
| Terlipressin (triglycyl lycine vasopressin) (CAS-14636-12-5)         | Analog of vasopressin; induces vasoconstriction.  | Terlipressin activity can be measured using assays of vasoconstriction, such as the isolated arterial ring preparation. (Landstrom et al., Hum Reprod 1999 Jan;14(1):151-5). | Variceal hemorrhage; cirrhosis; portal hypertension; hepatorenal syndrome; Blood-related disorders   |              | SEQ ID NO: 2202       |
| Ularitide (CAS-118812-69-4)  | Stimulates natriuresis, diuresis, and vasodilation.   | Ularitide activity can be assessed by measuring cGMP accumulation in rat renal cells. (Valentin et al., Hypertension 1993 Apr;21(4):432-8).                                  | Excretory disorders; Acute renal failure; asthma; congestive heart failure; hypertension; pulmonary hypertension; cardiovascular disorders |              | SEQ ID NO: 2203       |
| Aprotinin (Trasylol) (CAS-9087-70-1; CAS-11061-94-2; CAS-12407-79-3) | Serine protease inhibitor; attenuates Systemic Inflammatory Response, fibrinolysis and thrombin-induced platelet aggregation.   | Inhibition of thrombin-induced platelet aggregation can be measured using methods known in the art. (Poullis et al., J Thorac Cardiovasc Surg 2000 Aug;120(2):370-8).        | Inhibition of fibrinolysis; reduction of blood loss during surgery; Treatment of Inflammation and Immune Disorders.                        |              | SEQ ID NO: 2204       |
| Aspartocin (CAS-4117-65-1; CAS-1402-89-7)                            | Antibacteria  | Aspartocin activity can be assessed using in vitro antibacterial assays known in the art. (Zaslloff et al., Antimicrobial Agents and Chemotherapy 1999, 43:782-788).         | Treatment of Infectious Diseases; treatment of bacterial infections.   |              | SEQ ID NO: 2205       |



**Table 1**

| Therapeutic Protein:X   | Biological Activity   | Exemplary Activity Assay  | Preferred Indication: Y  | Construct ID | Therapeutic Protein:Z |
|---|---|---|--|--------------|-----------------------|
| Calcitonin (Calcimar) (CAS-21215-62-3)  | Regulates levels of calcium and phosphate in serum; causes a reduction in serum calcium--an effect opposite to that of human parathyroid hormone. | Hypocalcemic Rat Bioassay, bone resorbing assay and the pit assay, CT receptor binding assay, CAMP stimulation assay: J Bone Miner Res 1999 Aug;14(8):1425-31   | Musculoskeletal; Osteoporosis; Paget's disease; hypercalcemia; Bone Disorders; Fracture prevention; Malignant hypercalcemia; Osteopenia, Osteoclastogenesis; osteolysis; osteomyelitis; osteonecrosis; periodontal bone loss; osteoarthritis; rheumatoid arthritis; osteopetrosis; periodontal, lytic, or metastatic bone disease; osteoclast differentiation inhibition; bone disorders; bone healing and regeneration. |              | SEQ ID NO: 2206       |
| Carperitide (HANP; recombinant human atrial natriuretic peptide) (CAS-89213-87-6) | Stimulates natriuresis, diuresis, and vasodilation.   | Carperitide activity can be assessed in vitro by measuring cGMP accumulation in a number of cell lines, including PC12 cells and cultured human glomerular cells. (Medvede et al., Life Sci 2001 Aug 31;69(15):1783-90; Green et al., J Am Soc Nephrol 1994 Oct;5(4):1091-8). | Treatment of Heart Failure; Cardiovascular disorders; Respiratory disorders; Acute respiratory distress syndrome.  |              | SEQ ID NO: 2207       |
| Desirudin (recombinant hirudin; Revasc) (CAS-120993-53-5)                         | Inhibits thrombin; inhibits blood clotting.   | Desirudin activity can be assessed using blood clotting assays known in the art, such as in vitro platelet aggregation assays. (Glusa, Haemostasis 1991;21 Suppl 1:116-20).   | Blood-related disorder; Thrombosis; thrombocytopenia; hemorrhages.   |              | SEQ ID NO: 2208       |

**Table 1**

| Therapeutic Protein:X                       | Biological Activity   | Exemplary Activity Assay   | Preferred Indication: Y   | Construct ID | Therapeutic Protein:Z |
|---|---|--|---|--------------|-----------------------|
| Emoctakin (interleukin 8) (CAS-142298-00-8) | proinflammatory cytokine  |  | Treatment of Inflammation, Immune disorders, RSV infection.   |              | SEQ ID NO: 2209       |
| Felypressin (CAS-56-59-7)                   | Derivative of Vasopressin; Stimulates vasoconstriction; Induces local anesthesia. | Felypressin vasoconstriction activity can be measured using assays of vasoconstriction, such as the isolated arterial ring preparation. (Landstrom et al., Hum Reprod 1999 Jan;14(1):151-5). | Treatment of pain; to induce local anesthesia.  |              | SEQ ID NO: 2210       |
| Glucagon (CAS-16941-32-5)                   | Induces hyperglycemia.  | Glucagon activity may be assayed in vitro using a [3-H]-glucose uptake assay. (J Biol Chem 1999 Oct 22; 274(43):30864-30873).  | Hypoglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin-dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X; Endocrine disorders. |              | SEQ ID NO: 2211       |
| Nagrestipen (CAS-166089-33-4)               |   |  | Inflammation; Immune  |              | SEQ ID NO: 2212       |

**Table 1**

| <b>Therapeutic Protein:X</b>                                  | <b>Biological Activity</b>   | <b>Exemplary Activity Assay</b>   | <b>Preferred Indication: Y</b>   | <b>Construct ID</b> | <b>Therapeutic Protein:Z</b> |
|---|--|---|--|---------------------|------------------------------|
| Pentigetide (Pentyde) (CAS-62087-72-3)                        |  |   | Respiratory; Allergy; Immune   |                     | SEQ ID NO: 2213              |
| Proinsulin (CAS-67422-14-4)                                   | Stimulates glucose uptake and promotes glycogenesis and lipogenesis. | Insulin activity may be assayed in vitro using a [ <sup>3</sup> -H]-glucose uptake assay. (J Biol Chem 1999 Oct 22; 274(43):30864-30873).   | Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin-dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X.  |                     | SEQ ID NO: 2214              |
| Becaplermin (Regranex; recombinant PDGF-BB) (CAS-165101-51-9) | Promotes wound healing.  | Becaplermin activity can be assessed using animal wound healing models known in the art. (Saba et al., Ann Plast Surg 2002 Jul;49(1):62-6). | Stimulate Epithelial Cell Proliferation; Stimulate Basal Keratinocytes; Promote Wound Healing; Stimulate Hair Follicle Production; Healing Of Dermal Wounds. Wound Healing; Eye Tissue Wounds, Dental Tissue Wounds, Oral Cavity Wounds, Diabetic Ulcers, Dermal Ulcers, Cubitus Ulcers, Arterial Ulcers, Venous Stasis Ulcers, Burns Resulting From Heat Exposure Or Chemicals, or Other Abnormal Wound Healing Conditions such as Uremia, Malnutrition, Vitamin Deficiencies or Complications Associated With Systemic Treatment With Steroids, Radiation Therapy or Antineoplastic Drugs or Antimetabolites; Promote Dermal |                     | SEQ ID NO: 2215              |

**Table 1**

| Therapeutic Protein:X | Biological Activity | Exemplary Activity Assay | Preferred Indication: Y   | Construct ID | Therapeutic Protein:Z |
|-----------------------|---------------------|--------------------------|---|--------------|-----------------------|
|                       |                     |                          | Reestablishment Subsequent To Dermal Loss; Increase the Adherence Of Skin Grafts To A Wound Bed; Stimulate Re-Epithelialization from The Wound Bed; To Promote Skin Strength; Improve The Appearance Of Aged Skin; Proliferate Hepatocytes, Lung, Breast, Pancreas, Stomach, Bladder, Small Intestine, Large Intestine; Sebocytes, Hair Follicles, Type II Pneumocytes, Mucin-Producing Goblet Cells, or Other Epithelial Cells, Endothelial Cells, Keratinocytes, or Basal Keratinocytes (and Their Progenitors) Contained Within The Skin, Lung, Liver, Bladder, Eye, Salivary Glands, or Gastrointestinal Tract; Reduce The Side Effects Of Gut Toxicity That Result From Radiation, |              |                       |

**Table 1**

| Therapeutic Protein:X | Biological Activity | Exemplary Activity Assay | Preferred Indication: Y   | Construct ID | Therapeutic Protein:Z |
|-----------------------|---------------------|--------------------------|---|--------------|-----------------------|
|                       |                     |                          | Chemotherapy Treatments Or Viral Infections; Cytoprotector, especially of the Small Intestine Mucosa or Bladder; Mucositis (Mouth Ulcers); Regeneration Of Skin; Full and/or Partial Thickness Skin Defects, including Burns, (e.g., Repopulation Of Hair Follicles, Sweat Glands, And Sebaceous Glands); Psoriasis; Epidermolysis Bullosa; Blisters; Gastric and/or Duodenal Ulcers; Reduce Scarring; Inflammatory Bowel Diseases; Crohn's Disease; Ulcerative Colitis; Gut Toxicity; Lung Damage; Repair Of Alveoli And/or Bronchiolar Epithelium; Acute Or Chronic Lung Damage; Emphysema, ARDS; Inhalation Injuries; Hyaline Membrane Diseases; Infant Respiratory Distress Syndrome; |              |                       |

**Table 1**

| Therapeutic Protein:X                    | Biological Activity  | Exemplary Activity Assay  | Preferred Indication: Y  | Construct ID | Therapeutic Protein:Z |
|--|--|---|--|--------------|-----------------------|
|  |  |   | Bronchopulmonary Displasia In Premature Infants; Fulminant Liver Failure; Cirrhosis, Liver Damage caused by Viral Hepatitis and/or Toxic Substances; Diabetes Mellitus; Inflammation; Cancer; Digestive disorders.   |              |                       |
| Ghrelin (Genbank Accession No. AB029434) | Stimulates release of growth hormone from anterior pituitary. Stimulates appetite and reduces fat burning. | Appetite and food intake can be measured by methods known in the art (Batterham et al. Nature 2002; 418:650654) | Endocrine; loss of body weight; loss of body weight associated with cancer or anorexia nervosa; loss of appetite; excessive appetite; body weight gain; Obesity; Diabetes; Acromegaly; Growth failure; Growth hormone deficiency; Growth failure and growth retardation Prader-Willi syndrome in children 2 years or older; Growth deficiencies; Growth failure associated with chronic renal insufficiency; Postmenopausal osteoporosis; burns; cachexia; |              | SEQ ID NO: 2216       |

**Table 1**

| Therapeutic Protein:X  | Biological Activity   | Exemplary Activity Assay  | Preferred Indication:Y  | Construct ID | Therapeutic Protein:Z |
|--|---|---|---|--------------|-----------------------|
|  |   |   | cancer cachexia; dwarfism; metabolic disorders; obesity; renal failure; Turner's Syndrome, pediatric and adult; fibromyalgia; fracture treatment; frailty, AIDS wasting   |              |                       |
| Ghrelin -binding antibody including antibody fragment, or dominant-negative form of Ghrelin receptor | Inhibits growth hormone release in response to Ghrelin; inhibits increase in appetite.                                | Appetite and food intake can be measured by methods known in the art (Batterham et al. Nature 2002; 418:650654)                               | Endocrine; Obesity; Diabetes; body weight gain; excessive appetite; loss of appetite; loss of body weight.  |              |                       |
| NOGO-66 peptide fragment (Genbank Accession No. NP_008939 (amino acids 62-101))                      |   |   | Neurodegenerative disorders; spinal cord injury; neuronal injury; brain trauma; stroke; multiple sclerosis; demyelinating disorders; neural activity and neurological diseases; neural cell (e.g., neuron, glial cell, and schwann cell) regeneration and/or growth |              | SEQ ID NO: 2217       |
| Gastric inhibitory polypeptide (GIP), including GIP fragments (Genbank Accession No. NM_004123)      | Increases nutrient uptake and tryglyceride accumulation in adipocytes, which leads to obesity and insulin resistance. | Nutrient uptake and tryglyceride accumulation can be measured by methods described in Miyawaki et al., Nat. Medicine, 2002, Vol 8(7):738-742. | Most preferred: loss of body weight, AIDS wasting, cachexia, loss of appetite. Other: Obesity; Diabetes; insulin resistance; body weight gain; excessive appetite.  |              | SEQ ID NO: 2218       |
| Gastric inhibitory polypeptide antibody, or antibody fragments                                       | Increased use of fat as predominant energy source; decreased accumulation of fat in adipocytes.                       | Fat utilization as an energy source can be measured as described in Miyawaki et al., Nat. Medicine, 2002, Vol 8(7):738-742.                   | Obesity; Diabetes; Insulin resistance; body weight gain.  |              |                       |

**Table 1**

| <b>Therapeutic Protein:X</b>   | <b>Biological Activity</b>  | <b>Exemplary Activity Assay</b>   | <b>Preferred Indication: Y</b>   | <b>Construct ID</b> | <b>Therapeutic Protein:Z</b> |
|--|---|---|--|---------------------|------------------------------|
| Gastric inhibitory peptide receptor or receptor fragments or variants including soluble fragments or variants (Genbank Accession Number NM_000164)   | Increased use of fat as predominant energy source; decreased accumulation of fat in adipocytes.   | Fat utilization as an energy source can be measured as described in Miyawaki et al., Nat. Medicine, 2002, Vol 8(7):738-742. | Most preferred: Obesity; Diabetes; body weight gain; excessive appetite; insulin resistance.<br>Other: loss of body weight, AIDS wasting, loss of appetite.  |                     | SEQ ID NO: 2219              |
| POMC (proopiomelanocortin), including fragments or variants (such as, for example, alpha-melanocyte stimulating hormone, $\alpha$ MSH, gamma melanocyte stimulating hormone, $\gamma$ MSH, beta-melanocyte stimulating hormone, $\beta$ MSH, adrenocorticotropin, ACTH, beta-endorphin, met-enkephalin) (Genbank Accession No. | Activity of POMC-derived fragments are diverse, and well-known in the art.<br><br>See, for example, Hadley et al., Ann N Y Acad Sci 1999 Oct 20;885:1-21; Dore, Prog Clin Biol Res 1990;342:22-7; Blalock, Ann N Y Acad Sci. 1999 Oct 20;885:161-72). |   | Preferred: resistance to stress; anti-inflammatory activity; analgesic activity; increased skin pigmentation; increased protein catabolism; increased gluconeogenesis; obesity; diabetes.<br>Other: decreased protein catabolism, decreased skin pigmentation, Addison's disease, Cushing's syndrome |                     | SEQ ID NO: 2220              |



**Table 1**

| Therapeutic Protein:X                   | Biological Activity  | Exemplary Activity Assay  | Preferred Indication: Y  | Construct ID | Therapeutic Protein:Z |
|---|--|---|--|--------------|-----------------------|
| NM_000930)                              |  |   |  |              |                       |
| HP 467, HP228 (US Patent No. 6,350,430) | See US Patent No. 6,350,430  | See US Patent No. 6,350,430   | Resistance to stress; anti-inflammatory activity; analgesic activity; increased skin pigmentation; increased protein catabolism; increased gluconeogenesis.  |              | SEQ ID NO: 2221       |
| NDP (US Patent No. 6,350,430)           | See US Patent No. 6,350,430  | See US Patent No. 6,350,430   | Resistance to stress; anti-inflammatory activity; analgesic activity; increased skin pigmentation; increased protein catabolism; increased gluconeogenesis.  |              | SEQ ID NO: 2222       |
| Interleukin-21 (IL-21)                  | Immunomodulator; inhibits interferon gamma production by Th1 cells.          | IL-21 activity can be assessed by measuring interferon gamma production in Th1 cells. (Wurster et al., J Exp Med 2002 Oct 7;196(7):969-77)                        | Autoimmune disorders; Inflammatory disorders; Treatment of Psoriasis; Rheumatoid Arthritis; Inflammatory bowel disease.  | 3298         | SEQ ID NO: 2177       |
| Interleukin-4 (IL-4)                    | Immunomodulator; promotes the differentiation of T cells into Th2 phenotype. | IL-4 activity can be assessed by measuring Th1 / Th2 cytokine responses of isolated spleen cells in vitro. (Waltz et al., Horm Metab Res 2002 Oct;34(10):561-9).  | Treatment of Psoriasis; Autoimmune disorders; Rheumatoid Arthritis; Inflammatory bowel disease; Inflammatory disorders.  | 3307         | SEQ ID NO: 2178       |
| Osteoclast Inhibitory Lectin (OCIL)     | Inhibits osteoclast formation.   | Osteoclast Inhibitory Lectin activity can be assessed using osteoclast formation assays known in the art. (Zhou et al., J Biol Chem 2002 Dec 13;277(50):48808-15) | Treatment of Bone Disorders; Osteoporosis; Fracture prevention; Hypercalcemia; Malignant hypercalcemia; Paget's disease; Osteopenia, Osteoclastogenesis; osteolysis; osteomyelitis; osteonecrosis; periodontal bone loss; osteoarthritis; rheumatoid arthritis; osteopetrosis; periodontal, lytic, or metastatic bone disease; osteoclast differentiation inhibition; bone healing | 3312         | SEQ ID NO: 2181       |

**Table 1**

| Therapeutic Protein:X | Biological Activity | Exemplary Activity Assay | Preferred Indication:Y | Construct ID | Therapeutic Protein:Z |
|-----------------------|---------------------|--------------------------|------------------------|--------------|-----------------------|
|                       |                     |                          | and regeneration.      |              |                       |

**Table 2**

| Fusion No. | Construct ID | Construct Name         | Description  | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence |
|------------|--------------|------------------------|--|-------------------|-------------|-------------|-------------|-------------|-------------|-----------------|
| 1          | 1520         | pC4:HSA/TR6.V30-H300   | Amino acids V30 to H300 of TR6 (fragment shown as V1 to H271 of SEQ ID NO:433) fused downstream of HSA.  | pC4               | 217         | 1           | 433         | 649         | 650         | HSA             |
| 2          | 1537         | pYPG:HSA.TR6coV30-E294 | Amino acids V30 to E294 of TR6 (fragment shown as V1 to E265 of SEQ ID NO:434) fused downstream of HSA. DNA encoding TR6 has been codon optimized. | pYPGaf            | 218         | 2           | 434         | 651         | 652         | HSA             |
| 3          | 1545         | pYPG:HSA.TR6coV30-L288 | Amino acids V30 to L288 of TR6 (fragment shown as V1 to L259 of SEQ ID NO:435) fused downstream of HSA. DNA encoding TR6 has been codon optimized. | pYPGaf            | 219         | 3           | 435         | 653         | 654         | HSA             |
| 4          | 1546         | pYPG:HSA.TR6coV30-R284 | Amino acids V30 to R284 of TR6 (fragment shown as V1 to R255 of SEQ ID NO:436) fused downstream of HSA. DNA encoding TR6 has been codon optimized. | pYPGaf            | 220         | 4           | 436         | 655         | 656         | HSA             |
| 5          | 1568         | pSAC35:HSA-yTR6        | TR6 fused downstream of HSA. DNA encoding TR6 has been codon optimized.  | pSAC35            | 221         | 5           | 437         | 657         | 658         | HSA/kex2        |
| 6          | 1570         | pSAC35:TR6-HSA         | Mature TR6 fused downstream of the HSA/kex2 leader and upstream of the mature HSA.   | pSAC35            | 222         | 6           | 438         | 659         | 660         | HSA/kex2        |
| 7          | 1622         | pC4:synTR6.M1-H300.HSA | Synthetic TR6 fused upstream of mature HSA, with 2 extra amino acids between the TR6 and HSA portions.   | pC4               | 223         | 7           | 439         | 661         | 662         | Native TR6      |

**Table 2**

| Fusion No. | Construct ID | Construct Name             | Description   | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence |
|------------|--------------|----------------------------|---|-------------------|--------------|--------------|--------------|--------------|--------------|-----------------|
| 8          | 1623         | pC4:HSA:synTR6.V30-H300    | Synthetic mature TR6 fused downstream of FL HSA. Last amino acid HSA sequence is missing at BSU361 site.  | pC4               | 224          | 8            | 440          | 663          | 664          | HSA             |
| 9          | 1642         | pSAC35:GCSF.T31-P204.HSA   | Mature GCSF cloned downstream of the HSA/kex2 leader and upstream of the mature HSA   | pSAC35            | 225          | 9            | 441          | 665          | 666          | HSA/kex2        |
| 10         | 1643         | pSAC35:HSA.GCSF.T31-P204   | Mature GCSF cloned downstream of the mature HSA and HSA/kex2 leader sequence.   | pSAC35            | 226          | 10           | 442          | 667          | 668          | HSA/kex2        |
| 11         | 1645         | pSAC35:yTR6(N173Q).HSA     | Mutant mature TR6 cloned upstream of mature HSA and downstream of the HSA/kex2 leader sequence.   | pSAC35            | 227          | 11           | 443          | 669          | 670          | HSA/kex2        |
| 12         | 1657         | pC4.HSA:DeCAF.A23-D233     | Amino acids A23 to D233 of DeCAF fused downstream of full length HSA.   | pC4               | 228          | 12           | 444          | 671          | 672          | HSA             |
| 13         | 1680         | pYPG:HSA.BLyS.A134-L285    | Amino acids A134 to L285 of BLyS fused downstream of FL HSA. Two extra amino acids (Leu, Glu) have been added between the therapeutic protein and HSA portions. | pYPGaf            | 229          | 13           | 445          | 673          | 674          | HSA             |
| 14         | 1681         | pYPG.HSA.MPIF.D45-N120     | Amino acids D45 to N120 of MPIF fused downstream of FL HSA. Two additional amino acids (L and E) have been added between HSA and MPIF.                          | pYPGaf            | 230          | 14           | 446          | 675          | 676          | HSA             |
| 15         | 1697         | pSAC35:HSA.GM-CSF.A18-E144 | Amino acids A18 to E144 of GM-CSF fused downstream of FL HSA.   | pSAC35            | 231          | 15           | 447          | 677          | 678          | HSA             |

**Table 2**

| Fusion No. | Construct ID | Construct Name             | Description  | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence |
|------------|--------------|----------------------------|--|-------------------|--------------|--------------|--------------|--------------|--------------|-----------------|
| 16         | 1699         | pSAC35:GM-CSF.A18-E144:HSA | Amino acids A18 to E144 of GM-CSF fused upstream of mature HSA and downstream of HSA/kex2 leader.  | pSAC35            | 232          | 16           | 448          | 679          | 680          | HSA/kex2        |
| 17         | 1700         | pSAC35:HSA-yTR6(N173Q)     | Mutant TR6 fused downstream of mature HSA with HSA/kex2 leader sequence.   | pSAC35            | 233          | 17           | 449          | 681          | 682          | HSA/kex2        |
| 18         | 1702         | pYPG:HSA.ek.TR6coV 30-L288 | Amino acids V30 to L288 of TR6 (fragment shown as V1 to L259 of SEQ ID NO:450) fused downstream of FL HSA with an enterokinase site in between. DNA encoding TR6 has been codon optimized. | pYPGaf            | 234          | 18           | 450          | 683          | 684          | HSA             |
| 19         | 1703         | pYPG:HSA.ek.TR6coV 30-R284 | Amino acids V30 to R284 of TR6 (fragment shown as V1 to R255 of SEQ ID NO:451) fused downstream of HSA with an enterokinase site in between. DNA encoding TR6 has been codon optimized.    | pYPGaf            | 235          | 19           | 451          | 685          | 686          | HSA             |
| 20         | 1704         | pYPG:HSA.TR6.V30-E294      | Amino acids V30 to E294 of TR6 fused downstream of HSA. Two additional amino acids (Leu, Glu) are in between HSA and TR6.  | pYPGaf            | 236          | 20           | 452          | 687          | 688          | HSA             |
| 21         | 1746         | pYPG:HSA.ek.KDIL28-K207    | Amino acids L28 to K207 of KDI fused downstream of HSA with an enterokinase site in between.   | pYPGaf            | 237          | 21           | 453          | 689          | 690          | HSA             |
| 22         | 1747         | pSAC35:HSA.hGHRF.Y32-L75   | Amino acids Y32 to L75 of hGHRF fused downstream of HSA.   | pSAC35            | 238          | 22           | 454          | 691          | 692          | HSA             |

**Table 2**

| Fusion No. | Construct ID | Construct Name                 | Description  | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence |
|------------|--------------|--------------------------------|--|-------------------|--------------|--------------|--------------|--------------|--------------|-----------------|
| 23         | 1748         | pSAC35:hGHRF.Y32-L75.HSA       | Amino acids Y32 to L75 of hGHRF (see also SEQ IDNO:454) fused upstream of mature HSA and downstream of HSA/kex2 leader sequence.   | pSAC35            | 239          | 23           | 455          | 693          | 694          | HSA/kex2        |
| 24         | 1749         | pSAC35:HSA.PTH.S1-F3           | FL HSA fused upstream of amino acids S1-F34 of PTH   | pSAC35            | 240          | 24           | 456          | 695          | 696          | HSA             |
| 25         | 1750         | pSAC35:PTH.S1-F34.HSA          | Amino acids 1-34 of PTH fused upstream of mature HSA and downstream of HSA/kex2 leader sequence.   | pSAC35            | 241          | 25           | 457          | 697          | 698          | HSA/kex2        |
| 26         | 1757         | pSAC35:IL2.A21-T153.145C/S.HSA | Mature human IL-2 with a single amino acid mutation (C to S at position 145) cloned downstream of the HSA/KEX2 leader and upstream of mature HSA   | pSAC35            | 242          | 26           | 458          | 699          | 700          | HSA/kex2        |
| 27         | 1758         | pSAC35:HSA.IL2.A21-T153.145C/S | Mature human IL-2 with a single amino acid mutation (C to S at position 145) cloned downstream of HSA with HSA/kex2 leader sequence.   | pSAC35            | 243          | 27           | 459          | 701          | 702          | HSA/kex2        |
| 28         | 1772         | pSAC:EPOco.A28-D192.HSA        | Amino acids A28-D192 of EPO variant (where glycine at amino acid 140 has been replaced with an arginine) fused upstream of mature HSA and downstream of HSA/kex2 leader sequence. DNA encoding EPO has been codon optimized. | pSAC35            | 244          | 28           | 460          | 703          | 704          | HSA/kex2        |

**Table 2**

| Fusion No. | Construct ID | Construct Name                            | Description   | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence |
|------------|--------------|---|---|-------------------|-------------|-------------|-------------|-------------|-------------|-----------------|
| 29         | 1774         | pSAC:HSA:EPOco.A28-D192.                  | Amino acids A28-D192 of EPO variant (where glycine at amino acid 140 has been replaced with an arginine) fused downstream of HSA with HSA/kex2 leader sequence. DNA encoding EPO has been codon optimized.  | pSAC35            | 245         | 29          | 461         | 705         | 706         | HSA/kex2        |
| 30         | 1777         | pSAC35:TNFR2.L23-D257.HSA                 | Mature TNFR2 fused downstream of the HSA/kex2 signal and upstream of mature HSA.  | pSAC35            | 246         | 30          | 462         | 707         | 708         | HSA/kex2        |
| 31         | 1778         | pSAC35:IFN $\beta$ .M22-N187:HSA          | Residues M22-N187 of full-length IFN $\beta$ (shown as M1 to N166 of SEQ ID NO:463) fused upstream of mature HSA and downstream of HSA/kex2 leader sequence.  | pSAC35            | 247         | 31          | 463         | 709         | 710         | HSA/kex2        |
| 32         | 1779         | pSAC35:HSA:IFN $\beta$ .M22-N187          | Residues M22-N187 of full-length IFN $\beta$ (shown as M1 to N166 of SEQ ID NO:464) fused downstream of HSA with HSA/kex2 leader sequence.  | pSAC35            | 248         | 32          | 464         |             |             | HSA/kex2        |
| 33         | 1781         | pSAC:EPOcoA28-D192.HSA 51N/S,65N/S,110N/S | Amino acids A28-D192 of EPO variant (where glycine at amino acid 140 has been replaced with an arginine) fused upstream of mature HSA and downstream of HSA/kex2 leader sequence. Glycosylation sites at amino acid 51, 65, 110 are mutated from N to S residue. DNA encoding EPO has been codon optimized. | pSAC35            | 249         | 33          | 465         | 711         | 712         | HSA/kex2        |

**Table 2**

| Fusion No. | Construct ID | Construct Name                             | Description  | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence |
|------------|--------------|--|--|-------------------|-------------|-------------|-------------|-------------|-------------|-----------------|
| 34         | 1783         | pSAC:HSA.EPOcoA28-D192.5IN/S,65N/S,110 N/s | Amino acids A28-D192 of EPO variant (where glycine at amino acid 140 has been replaced with an arginine) fused downstream of HSA with HSA/kex2 leader sequence. Glycosylation sites at amino acids 51, 65, 110 are mutated from N to S residue. DNA encoding EPO has been codon optimized. | pSAC35            | 250         | 34          | 466         | 713         | 714         | HSA/kex2        |
| 35         | 1784         | pSAC35:HSA.TNFR2.L23-D257                  | Mature TNFR2 fused downstream of FL HSA.   | pSAC35            | 251         | 35          | 467         | 715         | 716         | HSA             |
| 36         | 1785         | pSAC35:KGF2Δ28.A63-S208:HSA                | Amino acids A63 to S208 of KGF2 fused upstream of mature HSA and downstream of the HSA/kex2 signal peptide.  | pSAC35            | 252         | 36          | 468         | 717         | 718         | HSA/kex2        |
| 37         | 1786         | pSAC35:HSA.KGF2{D}28.A63-S208              | Amino acids A63 to S208 of KGF2 fused downstream of HSA.   | pSAC35            | 253         | 37          | 469         | 719         | 720         | HSA             |
| 38         | 1788         | pSAC35:HSA.TR2.P37-A192                    | Amino acids P37 to A192 of TR2 fused downstream of HSA with HSA/kex2 leader sequence.  | pSAC35            | 254         | 38          | 470         | 721         | 722         | HSA/kex2        |
| 39         | 1793         | pSAC35:HSA.EPO.A28-D192 (N51A,N65A,N110A)  | Amino acids A28-D192 of EPO variant (where glycine at amino acid 140 has been replaced with an arginine; see, for example, SEQ ID NO:499) fused downstream of HSA with HSA/kex2 leader sequence. Glycosylation sites at amino acids 51, 65, 110 are mutated from N to A residue.           | pSAC35            | 255         | 39          | 471         |             |             | HSA/kex2        |



**Table 2**

| Fusion No. | Construct ID | Construct Name                   | Description  | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence                                     |
|------------|--------------|----------------------------------|--|-------------------|--------------|--------------|--------------|--------------|--------------|---|
| 40         | 1794         | pSAC35:HSA.EPO.A28-D192          | Amino acids A28-D192 of the EPO variant (where glycine at amino acid 140 has been replaced with an arginine; see, for example, SEQ ID NO:499) fused downstream of HSA with HSA/kex2 leader sequence. | pSAC35            | 256          | 40           | 472          |              |              | HSA/kex2  |
| 41         | 1809         | pSAC35:MDC.G25-Q93.HSA           | Amino acids P26 to Q93 of MDC with an N-terminal methionine, fused downstream of the HSA/kex2 leader and upstream of mature HSA.   | pSAC35            | 257          | 41           | 473          | 723          | 724          | HSA/kex2  |
| 42         | 1812         | pSAC35:IL2.A21-T153.HSA          | Amino acids A21 to T153 of IL-2 fused downstream of the HSA/kex2 leader and upstream of mature HSA.  | pSAC35            | 258          | 42           | 474          | 725          | 726          | HSA/kex2  |
| 43         | 1813         | pSAC35:HSA.IL2.A21-T153          | Amino acids A21 to T153 of IL-2 fused downstream of HSA with HSA/kex2 leader sequence.   | pSAC35            | 259          | 43           | 475          | 727          | 728          | HSA/kex2  |
| 44         | 1821         | pSAC35:scFv116A01.HSA            | BLyS antibody fused upstream of mature HSA which lacks the first 8 amino acids and downstream from the HSA/kex2 signal sequence which lacks the last two amino acids.                                | pSAC35            | 260          | 44           | 476          | 729          | 730          | Modified HSA/kex2, lacking the last two amino acids |
| 45         | 1830         | pSAC35:HSA.KEX2.HAGDG59.L19-Q300 | Amino acids L19-Q300 of HAGDG59 fused downstream of the HSA/kex2 signal, mature HSA and KEX2 cleavage site.  | pSAC35            | 261          | 45           | 477          | 731          | 732          | HSA/kex2  |

**Table 2**

| Fusion No. | Construct ID | Construct Name                     | Description   | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence |
|------------|--------------|------------------------------------|---|-------------------|-------------|-------------|-------------|-------------|-------------|-----------------|
| 46         | 1831         | pSAC35:HAGDGS9.L19-Q300.HSA        | HSA/kex2 signal peptide followed by amino acids L19-Q300 of HAGDGS9 followed by mature HSA.   | pSAC35            | 262         | 46          | 478         | 733         | 734         | HSA/kex2        |
| 47         | 1833         | pSAC35:humancalcitonin.C1-G33:HSA  | Human Calcitonin (amino acids C98-G130 of SEQ ID NO:479) fused upstream of mature HSA and downstream of HSA/kex2 leader sequence.         | pSAC35            | 263         | 47          | 479         | 735         | 736         | HSA/kex2        |
| 48         | 1834         | pSAC35:HSA.humancalcitonin.C1-G33  | Human Calcitonin (amino acids C98-G130 of SEQ ID NO:480) fused downstream of FL HSA.  | pSAC35            | 264         | 48          | 480         | 737         | 738         | HSA             |
| 49         | 1835         | pSAC35:salmoncalcitonin.C1-G33:HSA | Salmon Calcitonin amino acids C1-G33 fused upstream of mature HSA and downstream of HSA/kex2 leader sequence.                             | pSAC35            | 265         | 49          | 481         | 739         | 740         | HSA/kex2        |
| 50         | 1836         | pSAC35:HSA.salmoncalcitonin.C1-G33 | Salmon Calcitonin amino acids C1-G33 fused downstream of HSA.   | pSAC35            | 266         | 50          | 482         | 741         | 742         | HSA             |
| 51         | 1853         | pSAC35:PTH(1-34)N26.HSA            | Amino acids 1 to 34 of PTH fused upstream of mature HSA and downstream of HSA/kex2 leader sequence. Amino acid K26 of PTH mutated to N26. | pSAC35            | 267         | 51          | 483         | 743         | 744         | HSA/kex2        |
| 52         | 1854         | pSAC35:HSA.PTH(1-34)N26            | Amino acids 1 to 34 of PTH fused downstream of HSA. Amino acid K26 of PTH mutated to N26.   | pSAC35            | 268         | 52          | 484         | 745         | 746         | HSA             |
| 53         | 1862         | pSAC35:HSA.GnRH.Q24-G33            | Amino acids Q24-G33 of human gonadotropin releasing hormone fused downstream of HSA with HSA/kex2 leader sequence.                        | pSAC35            | 269         | 53          | 485         | 747         | 748         | HSA/kex2        |

**Table 2**

| Fusion No. | Construct ID | Construct Name                     | Description   | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence   |
|------------|--------------|------------------------------------|---|-------------------|--------------|--------------|--------------|--------------|--------------|-------------------|
| 54         | 1863         | pSAC35:GnRHQ24-G33.HSA             | Amino acids Q24-G33 of human gonadotropin releasing hormone fused upstream of mature HSA and downstream of HSA/kex2 leader sequence.                                    | pSAC35            | 270          | 54           | 486          | 749          | 750          | HSA/kex2          |
| 55         | 1866         | pSAC35:teprotide.HSA               | Teprotide fused upstream of mature HSA.   | pSAC35            | 271          | 55           | 487          | 751          | 752          |                   |
| 56         | 1867         | pSAC35:HSA.teprotide.              | Teprotide fused downstream of FL HSA.   | pSAC35            | 272          | 56           | 488          | 753          | 754          | HSA               |
| 57         | 1889         | pC4:HSA.PTH.S1-F34                 | PTH(1-34) fused downstream of HSA.  | pC4               | 273          | 57           | 489          | 755          | 756          | HSA               |
| 58         | 1891         | pEE12:HSA.sTR6                     | Soluble mature TR6 fused downstream of HSA.   | pEE12.1           | 274          | 58           | 490          | 757          | 758          | HSA               |
| 59         | 1892         | pEE12:sTR6.HSA                     | Synthetic full length TR6 fused upstream of mature HSA.   | pEE12.1           | 275          | 59           | 491          | 759          | 760          | TR6               |
| 60         | 1906         | pC4:PTH.S1-F34.HSA (junctioned)    | Amino acids S1 to F34 of PTH fused upstream of mature HSA and downstream of MPIF leader sequence. There are two cloning junction amino acids (T,S) between PTH and HSA. | pC4               | 276          | 60           | 492          | 761          | 762          | MPIF              |
| 61         | 1907         | pC4:HSA.PTH.S1-F34 (junctioned)    | Amino acids S1 to F34 fused downstream of FL HSA. The last C-terminal amino acid (L) residue is missing for HSA in the cloning junction between HSA and PTH.            | pC4               | 277          | 61           | 493          | 763          | 764          | HSA               |
| 62         | 1912         | pC4:sTR6.HSA                       | Synthetic full length TR6 fused upstream of mature HSA.   | pC4               | 278          | 62           | 494          | 765          | 766          | Native TR6 leader |
| 63         | 1913         | pC4:HSA.synTR6.V30-H300 (seamless) | Amino acids V30 to H300 of synthetic TR6 (shown as V1 to H271 of SEQ ID NO:495) fused downstream of full-length HSA.  | pC4               | 279          | 63           | 495          | 767          | 768          | HSA               |

**Table 2**

| Fusion No. | Construct ID | Construct Name                | Description  | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence           |
|------------|--------------|-------------------------------|--|-------------------|-------------|-------------|-------------|-------------|-------------|---------------------------|
| 64         | 1914         | pC4:PTH.S1-F34.HSA (seamless) | Amino acids S1 to F34 of PTH fused downstream of MPIF leader sequence and upstream of mature HSA.  | pC4               | 280         | 64          | 496         | 769         | 770         | MPIF                      |
| 65         | 1916         | pC4:HSA.KGF2D28.A63-S208      | Amino acids A63 to S208 of full length KGF2 fused downstream of HSA.   | pC4               | 281         | 65          | 497         | 771         | 772         | HSA                       |
| 66         | 1917         | pC4:KGF2D28.A63-S208:HSA      | Amino acids A63 to S208 of KGF2 fused upstream of mature HSA.  | pC4               | 282         | 66          | 498         | 773         | 774         | HSA/kex2                  |
| 67         | 1925         | pcDNA3:EPO M1-D192.HSA        | Amino acids M1 to D192 of EPO variant (where glycine at amino acid 140 has been replaced with an arginine) fused upstream of HSA. D192 of EPO and D1 of mature HSA are the same amino acids in this construct. | pcDNA3            | 283         | 67          | 499         | 775         | 776         | Native EPO leader peptide |
| 68         | 1926         | pcDNA3:SPHSA.EPO A28-D192     | Amino acids A28 to D192 of EPO variant (where glycine at amino acid 140 has been replaced with an arginine) fused upstream of mature HSA and downstream of the MPIF leader peptide.                            | pcDNA3            | 284         | 68          | 500         | 777         | 778         | MPIF                      |
| 69         | 1932         | pEE12.1:HSA.PTH.S1-F34        | Amino acids 1 to 34 of PTH fused downstream of full length HSA.  | pEE12.1           | 285         | 69          | 501         | 779         | 780         | HSA                       |
| 70         | 1933         | pSAC35:HCC-1.T20-N93:HSA      | Amino acids T20 to N93 of HCC-1 fused upstream of mature HSA and downstream of the HSA/kex2 leader sequence.   | pSAC35            | 286         | 70          | 502         | 781         | 782         | HSA/kex2                  |

**Table 2**

| Fusion No. | Construct ID | Construct Name                  | Description  | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence    |
|------------|--------------|---------------------------------|--|-------------------|--------------|--------------|--------------|--------------|--------------|--------------------|
| 71         | 1934         | pSAC35:HCC-1C.O.T20-N93:HSA     | Amino acids T20 to N93 of HCC-1 fused upstream of mature HSA and downstream of the HSA/kex2 leader sequence. DNA sequence is codon optimized for yeast expression.       | pSAC35            | 287          | 71           | 503          | 783          | 784          | HSA/kex2           |
| 72         | 1938         | pEE12.1:PTH.S1-F34.HSA          | Amino acids S1 to F34 of PTH fused upstream of mature HSA and downstream of MPIF leader sequence.  | pEE12.1           | 288          | 72           | 504          | 785          | 786          | MPIF               |
| 73         | 1941         | pC4:HSA/PTH84 (junctioned)      | PTH fused downstream of full length HSA. The last amino acid of HSA (Leu) has been deleted.  | pC4               | 289          | 73           | 505          | 787          | 788          | HSA                |
| 74         | 1947         | pSAC35:d8HCC-1.G28-N93:HSA      | Amino acids G28 to N93 of HCC-1 fused upstream of mature HSA and downstream of HSA/kex2 leader sequence.   | pSAC35            | 290          | 74           | 506          | 789          | 790          | HSA/kex2           |
| 75         | 1948         | pSAC35:d8HCC-1C.O.G28-N93:HSA   | Amino acids G28 to N93 of HCC-1 fused upstream of mature HSA and downstream of HSA/kex2 leader sequence. DNA sequence is codon optimized for yeast expression.           | pSAC35            | 291          | 75           | 507          | 791          | 792          | HSA/kex2           |
| 76         | 1949         | pC4:PTH.S1-Q84/HSA (junctioned) | PTH fused downstream of the MPIF leader sequence and upstream of mature HSA. There are two additional amino acids between PTH84 and HSA as a result of the cloning site. | pC4               | 292          | 76           | 508          | 793          | 794          | MPIF               |
| 77         | 1952         | pcDNA3.1:IL2.HSA                | Full length human IL-2, having a Cysteine to Serine mutation at amino acid 145, fused upstream of mature HSA.  | pcDNA3.1          | 293          | 77           | 509          | 795          | 796          | Native IL-2 leader |

**Table 2**

| Fusion No. | Construct ID | Construct Name  | Description  | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence           |
|------------|--------------|---|--|-------------------|--------------|--------------|--------------|--------------|--------------|---------------------------|
| 78         | 1954         | pC4:IL2.HSA   | Full length human IL-2, having a Cysteine to Serine mutation at amino acid 145, fused upstream of mature HSA.  | pC4               | 294          | 78           | 510          | 797          | 798          | Native IL-2 leader        |
| 79         | 1955         | pSAC35:t9HCC-1.G28-N93:spcHSA                                     | Amino acids G28 to N93 of HCC-1 fused upstream of a 16 amino acid spacer and mature HSA and downstream of HSA/kex2 leader sequence.  | pSAC35            | 295          | 79           | 511          | 799          | 800          | HSA/kex2                  |
| 80         | 1956         | pSAC35:HSA.scFv116 A01  | Single chain BLyS antibody fused downstream of HSA with HSA/kex2 leader sequence. This construct also contains a His tag at the 3' end.  | pSAC35            | 296          | 80           | 512          | 801          | 802          | HSA/kex2                  |
| 81         | 1966         | pC4:EPO.M1-D192.HSA<br>Construct is also named pC4:EPOM1-D192.HSA | Amino acids M1 to D192 of EPO variant (where glycine at amino acid 140 has been replaced with an arginine) fused upstream of mature HSA.   | pC4               | 297          | 81           | 513          |              |              | Native EPO leader peptide |
| 82         | 1969         | pC4:MPIFsp.HSA.EPO.A28-D192                                       | Amino acids A28 to D192 of EPO variant (where glycine at amino acid 140 has been replaced with an arginine) fused downstream of MPIF leader sequence and upstream of mature HSA.   | pC4               | 298          | 82           | 514          |              |              | MPIF                      |
| 83         | 1980         | pC4:EPO.A28-D192.HSA  | Amino acids A28 to D192 of EPO variant (where glycine at amino acid 140 has been replaced with an arginine) fused downstream of the HSA leader peptide and upstream of mature HSA. | pC4               | 299          | 83           | 515          | 803          | 804          | HSA                       |

**Table 2**

| Fusion No. | Construct ID | Construct Name                  | Description  | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence   |
|------------|--------------|---------------------------------|--|-------------------|--------------|--------------|--------------|--------------|--------------|-------------------|
| 84         | 1981         | pC4.HSA-EPO.A28-D192.           | Amino acids A28 to D192 of EPO variant (where glycine at amino acid 140 has been replaced with an arginine) fused downstream of the full length HSA.                               | pC4               | 300          | 84           | 516          | 805          | 806          | HSA               |
| 85         | 1989         | pSAC35:activeAC2inhibitor:HSA   | Active inhibitor of ACE2 (DX512) fused upstream of mature HSA and downstream of HSA/kex2 leader sequence.  | pSAC35            | 301          | 85           | 517          | 807          | 808          | HSA/kex2          |
| 86         | 1994         | pEE12.1.HSA-EPO.A28-D192.       | Amino acids A28 to D192 of EPO variant (where glycine at amino acid 140 has been replaced with an arginine) fused downstream of full length HSA.                                   | pEE12.1           | 302          | 86           | 518          |              |              | HSA               |
| 87         | 1995         | pEE12.1:EPO.A28-D192. HSA       | Amino acids A28 to D192 of EPO variant (where glycine at amino acid 140 has been replaced with an arginine) fused downstream of the HSA leader peptide and upstream of mature HSA. | pEE12.1           | 303          | 87           | 519          |              |              | HSA               |
| 88         | 1996         | pEE12.1:MPIFsp.HSA.EPO.A28-D192 | Amino acids A28 to D192 of EPO variant (where glycine at amino acid 140 has been replaced with an arginine) fused downstream of MPIF leader sequence and upstream of mature HSA.   | pEE12.1           | 304          | 88           | 520          |              |              | MPIF              |
| 89         | 1997         | pEE12.1:EPO M1-D192.HSA         | Amino acids M1 to D192 of EPO variant (where glycine at amino acid 140 has been replaced with an arginine) fused upstream of mature HSA.   | pEE12.1           | 305          | 89           | 521          |              |              | Native EPO leader |

**Table 2**

| Fusion No. | Construct ID | Construct Name                  | Description  | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence    |
|------------|--------------|---------------------------------|--|-------------------|-------------|-------------|-------------|-------------|-------------|--------------------|
| 90         | 1998         | pC4:CKB1.G28-N93.HSA            | Amino acids G28 to N93 of CkBeta1 fused upstream of mature HSA and downstream of the HSA leader sequence.                | pC4               | 306         | 90          | 522         | 809         | 810         | HSA                |
| 91         | 2000         | pSAC35:HSA:activeAC2inhibitor   | Active inhibitor of ACE2 (DX512) fused downstream of HSA.  | pSAC35            | 307         | 91          | 523         | 811         | 812         | HSA                |
| 92         | 2001         | pSAC35:inactiveAC2inhibitor:HSA | Inactive inhibitor of ACE2 (DX510) fused upstream of mature HSA and downstream of HSA/kex2 leader sequence.              | pSAC35            | 308         | 92          | 524         | 813         | 814         | HSA/kex2           |
| 93         | 2002         | pSAC35:HSA.inactiveAC2inhibitor | Inactive inhibitor of ACE2 (DX510) fused downstream of HSA.  | pSAC35            | 309         | 93          | 525         | 815         | 816         | HSA                |
| 94         | 2011         | pC4:IFNb-HSA                    | Full length IFNb fused upstream of mature HSA.   | pC4               | 310         | 94          | 526         | 817         | 818         | Native IFNb leader |
| 95         | 2013         | pC4:HSA-IFNb.M22-N187           | Amino acids M22 to N187 of IFNb (fragment shown as amino acids M1 to N166 of SEQ ID NO:527) fused downstream of HSA.     | pC4               | 311         | 95          | 527         |             |             | HSA                |
| 96         | 2016         | pC4:TR1.M1-L401.HSA             | Amino acids M1 to L401 of TR1 fused upstream of mature HSA. Native TR1 signal sequence used. A Kozak sequence was added. | pC4               | 312         | 96          | 528         | 819         | 820         | Native TR1         |
| 97         | 2017         | pC4:HSA.TR1.E22-L401            | Amino acids E22 to L401 of TR1 fused downstream of HSA.  | pC4               | 313         | 97          | 529         | 821         | 822         | HSA                |
| 98         | 2021         | pC4:PTH.S1-Q84/HSA (seamless)   | Amino acids 1-84 of PTH fused upstream of mature HSA and downstream of native HSA leader sequence.                       | pC4               | 314         | 98          | 530         | 823         | 824         | HSA                |



**Table 2**

| Fusion No. | Construct ID | Construct Name   | Description   | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence           |
|------------|--------------|--|---|-------------------|--------------|--------------|--------------|--------------|--------------|---------------------------|
| 99         | 2022         | pEE12.1:PTH.S1-Q84.HSA   | Amino acids 1-84 of PTH fused upstream of mature HSA and downstream of native HSA leader sequence.  | pEE12.1           | 315          | 99           | 531          |              |              | HSA                       |
| 100        | 2023         | pSAC35:PTH.S1-Q84.HSA  | Amino acids 1-84 of PTH fused upstream of mature HSA and downstream of HSA/kex2 leader sequence.  | pSAC35            | 316          | 100          | 532          | 825          | 826          | HSA/kex2                  |
| 101        | 2025         | pSAC35:teprotide.space r.HSA                                     | Teprotide fused upstream of a linker and mature HSA.  | pSAC35            | 317          | 101          | 533          | 827          | 828          |                           |
| 102        | 2026         | pSAC35:HSA.spacer.teprotide                                      | Teprotide fused downstream of HSA and a linker.   | pSAC35            | 318          | 102          | 534          | 829          | 830          | HSA                       |
| 103        | 2030         | pSAC35.ycoIL-2.A21-T153.HSA                                      | Amino acids A21 to T153 of IL-2 fused upstream of mature HSA and downstream of HSA/kex2 leader sequence. DNA encoding IL-2 has been codon optimized.                            | pSAC35            | 319          | 103          | 535          | 831          | 832          | HSA/kex2                  |
| 104        | 2031         | pSAC35.HSA.ycoIL-2.A21-T153                                      | Amino acids A21 to T153 of IL-2 fused downstream of HSA with the HSA/kex2 leader sequence. DNA encoding IL-2 has been codon optimized.  | pSAC35            | 320          | 104          | 536          | 833          | 834          | HSA/kex2                  |
| 105        | 2047         | pC4HSA:SP.EPO A28-D192.HSA                                       | Amino acids A28 to D192 of EPO variant (where glycine at amino acid 140 has been replaced with an arginine) fused upstream of mature HSA and downstream of MPIF leader peptide. | pSAC35            | 321          | 105          | 537          | 835          | 836          | MPIF                      |
| 106        | 2053         | pEE12:IFN $\beta$ -HSA<br>also named<br>pEE12.1:IFN $\beta$ -HSA | Full length IFN $\beta$ fused upstream of mature HSA.   | pEE12.1           | 322          | 106          | 538          |              |              | Native IFN $\beta$ leader |

**Table 2**

| Fusion No. | Construct ID | Construct Name              | Description   | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence     |
|------------|--------------|-----------------------------|---|-------------------|-------------|-------------|-------------|-------------|-------------|---------------------|
| 107        | 2054         | pEE12:HSA-IFN $\beta$       | Mature IFN $\beta$ fused downstream of HSA.   | pEE12.1           | 323         | 107         | 539         |             |             | HSA                 |
| 108        | 2066         | pC4:GM-CSF.M1-E144.HSA      | Amino acids M1 to E144 of GM-CSF fused upstream of mature HSA.  | pC4               | 324         | 108         | 540         | 837         | 838         | Native GM-CSF       |
| 109        | 2067         | pC4:HSA.GM-CSF.A18-E144     | Amino acids A18 to E144 of GM-CSF fused downstream of HSA.  | pC4               | 325         | 109         | 541         | 839         | 840         | HSA                 |
| 110        | 2085         | pEE12.1:TR1.M1-L401.HSA     | Amino acids M1 to L401 of TR1 fused upstream of mature HSA.   | pEE12.1           | 326         | 110         | 542         |             |             | Native TR-1         |
| 111        | 2086         | pEE12.1:HSA.TR1.E22-L401    | Amino acids E22 to L401 (fragment shown as amino acids E1 to L380 of SEQ ID NO:543) of TR1 fused downstream of HSA.   | pEE12.1           | 327         | 111         | 543         |             |             | HSA                 |
| 112        | 2095         | pC4:HSA-BLyS.A134           | Amino acids A134 to L285 of BLyS fused downstream of HSA.   | pC4               | 328         | 112         | 544         | 841         | 842         | HSA                 |
| 113        | 2096         | pC4:sp.BLyS.A134-L285.HSA   | Amino acids A134 to L285 of BLyS (fragment shown as amino acids A1 to L152 of SEQ ID NO:545) fused upstream of mature HSA and downstream of the CK $\beta$ 8 signal peptide.    | pC4               | 329         | 113         | 545         | 843         | 844         | Native CK $\beta$ 8 |
| 114        | 2101         | pcDNA3:SP.Ck7 Q22-A89.HSA.  | N-terminal Methionine fused to amino acids Q22 to A89 of Ck $\beta$ 7 fused upstream of mature HSA and downstream of MPIF signal peptide.                                       | pcDNA3            | 330         | 114         | 546         | 845         | 846         | MPIF                |
| 115        | 2102         | pEE12.1:SP:EPO A28-D192.HSA | Amino acids A28 to D192 of EPO variant (where glycine at amino acid 140 has been replaced with an arginine) fused upstream of mature HSA and downstream of MPIF leader peptide. | pEE12.1           | 331         | 115         | 547         |             |             | MPIF                |

**Table 2**

| Fusion No. | Construct ID | Construct Name                        | Description   | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence |
|------------|--------------|---------------------------------------|---|-------------------|--------------|--------------|--------------|--------------|--------------|-----------------|
| 116        | 2129         | pC4:TR2.M1-A192.HSA                   | Amino acids M1-A192 of TR2 fused upstream of HSA.   | pC4               | 332          | 116          | 548          | 847          | 848          | Native TR2      |
| 117        | 2137         | pSAC35.MDC.G25-Q93.HSA.               | Amino acids G25 to Q93 of MDC fused upstream of mature HSA and downstream of HSA/kex2 leader sequence.  | pSAC35            | 333          | 117          | 549          | 849          | 850          | HSA/kex2        |
| 118        | 2141         | HSA-CK-Beta4                          | Full length CK-beta4 fused downstream of HSA.   | pSAC35            | 334          | 118          | 550          | 851          | 852          | HSA             |
| 119        | 2146         | pC4:Leptin.HSA                        | Full length Leptin fused upstream of mature HSA.  | pC4               | 335          | 119          | 551          | 853          | 854          | Native leptin   |
| 120        | 2181         | pC4:HSA.IL1Ra(R8-E159)                | Amino acids R8 to E159 of IL1Ra (plus an added methionine at N-terminus) fused downstream of HSA.   | pC4               | 336          | 120          | 552          | 855          | 856          | HSA             |
| 121        | 2182         | pC4:MPIFsp(M1-A21).IL1Ra(R8-E159).HSA | Amino acids R8 to E159 of IL1Ra (plus an added methionine at N-terminus) fused downstream of the MPIF leader sequence and upstream of mature HSA. | pC4               | 337          | 121          | 553          | 857          | 858          | MPIF            |
| 122        | 2183         | pSAC35:HSA.IL1Ra(R8-E159)             | Amino acids R8 to E159 of IL1Ra (plus an added methionine at N-terminus) fused downstream of HSA.   | pSAC35            | 338          | 122          | 554          | 859          | 860          | HSA             |
| 123        | 2184         | pC4:HSA.Leptin.V22-C166               | Amino acids V22 to C167 of Leptin fused downstream of HSA.  | pC4               | 339          | 123          | 555          | 861          | 862          | HSA             |
| 124        | 2185         | pSAC35:IL1Ra(R8-E159).HSA             | Amino acids R8 to E159 of IL1Ra (plus an added methionine at N-terminus) fused upstream of mature HSA and downstream of HSA/kex2 leader sequence. | pSAC35            | 340          | 124          | 556          | 863          | 864          | HSA/kex2        |

**Table 2**

| Fusion No. | Construct ID | Construct Name               | Description   | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence |
|------------|--------------|------------------------------|---|-------------------|--------------|--------------|--------------|--------------|--------------|-----------------|
| 125        | 2186         | pSAC35:Leptin. V22-C166.HSA  | Amino acids V22 to C167 of Leptin fused upstream of mature HSA and downstream of HSA/kex2 leader sequence.  | pSAC35            | 341          | 125          | 557          | 865          | 866          | HSA/kex2        |
| 126        | 2187         | pSAC35:HSA.Leptin. V 22-C166 | Amino acids V22 to C167 of Leptin fused downstream of HSA with HSA/kex2 leader sequence.  | pSAC35            | 342          | 126          | 558          | 867          | 868          | HSA/kex2        |
| 127        | 2226         | pcDNA3(+):TREM-1(21-202)-HSA | Amino acids A21 to P202 of TREM-1 fused upstream of mature HSA and downstream of the MPIF leader sequence.  | pcDNA3.1          | 343          | 127          | 559          | 869          | 870          | MPIF            |
| 128        | 2230         | pC4:TREM-1.M1-P202.HSA       | Amino acids M1 to P202 of TREM-1 fused upstream of mature HSA.  | pC4               | 344          | 128          | 560          | 871          | 872          | Native TREM-1   |
| 129        | 2240         | pC4:SP.Ck7 Q22-A89.HSA.      | N-terminal Methionine fused to amino acids Q22 to A89 of Ckβ7 fused upstream of mature HSA and downstream of the MPIF leader sequence. Contains a linker sequence between Ckβ7 and HSA.         | pC4               | 345          | 129          | 561          | 873          | 874          | MPIF            |
| 130        | 2241         | pC4:HSA.Ck7metQ22-A89.       | N-terminal Methionine fused to amino acids Q22 to A89 of Chemokine beta 7 (Ckβ7 or CK7) fused downstream of HSA with HSA/kex2 leader sequence. Contains a linker sequence between Ckβ7 and HSA. | pC4               | 346          | 130          | 562          | 875          | 876          | HSA/kex2        |
| 131        | 2244         | pC4.HCNCA73.HSA              | HCNCA73 fused upstream of mature HSA.   | pC4               | 347          | 131          | 563          | 877          | 878          | HCNCA73         |

**Table 2**

| Fusion No. | Construct ID | Construct Name  | Description   | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence |
|------------|--------------|---|---|-------------------|-------------|-------------|-------------|-------------|-------------|-----------------|
| 132        | 2245         | pScNHSA:CK7.Q22-A89   | Amino acids Q22 to A89 of Ckβ7 fused downstream of HSA with HSA/kex2 leader sequence. Contains a linker sequence between Ckβ7 and HSA.                                | pScNHSA           | 348         | 132         | 564         | 879         | 880         | HSA/kex2        |
| 133        | 2246         | pScCHSA.CK7metQ22-A89   | N-terminal Methionine fused to amino acids Q22 to A89 of Ckβ7 fused upstream of mature HSA and downstream of HSA/kex2 leader sequence.                                | pScCHSA           | 349         | 133         | 565         | 881         | 882         | HSA/kex2        |
| 134        | 2247         | pSAC35:CK7metQ22-A89.HSA.   | N-terminal Methionine fused to amino acids Q22 to A89 of Ckβ7 fused upstream of mature HSA and downstream of HSA/kex2 leader sequence.                                | pSAC35            | 350         | 134         | 566         | 883         | 884         | HSA/kex2        |
| 135        | 2248         | pSAC35:HSA.CK7metQ22-A89.   | N-terminal Methionine fused to amino acids Q22 to A89 of Ckβ7 fused downstream of HSA with HSA/kex2 leader sequence. Contains a linker sequence between Ckβ7 and HSA. | pSAC35            | 351         | 135         | 567         | 885         | 886         | HSA/kex2        |
| 136        | 2249         | pSAC35:IFNα2-HSA<br>also named:<br>pSAC23:IFNα2-HSA                         | Mature IFNα2 fused upstream of mature HSA and downstream of HSA/kex2 leader sequence.   | pSAC35            | 352         | 136         | 568         | 887         | 888         | HSA/kex2        |
| 137        | 2250         | pSAC35:HSA.INSULIN (GYG)<br>also named:<br>pSAC35.HSA.INSULIN G(GYG).F1-N62 | Mature Insulin wherein the C-peptide is replaced by the C-domain of IGF-1 fused downstream of HSA. DNA encoding Insulin was codon optimized.                          | pSAC35            | 353         | 137         | 569         | 889         | 890         | HSA             |

**Table 2**

| Fusion No. | Construct ID | Construct Name  | Description   | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence |
|------------|--------------|---|---|-------------------|-------------|-------------|-------------|-------------|-------------|-----------------|
| 138        | 2251         | pScCHSA:VEGF2.T103-R227.  | Amino acids T103 to R227 of VEGF2 fused upstream of mature HSA and downstream of HSA/kex2 leader sequence.  | pScCHSA           | 354         | 138         | 570         | 891         | 892         | HSA/kex2        |
| 139        | 2252         | pScNHSA:VEGF2.T103-R227.  | Amino acids T103 to R227 of VEGF2 fused downstream of HSA with HSA/kex2 leader sequence.  | pScNHSA           | 355         | 139         | 571         | 893         | 894         | HSA/kex2        |
| 140        | 2255         | pSAC35:INSULIN(GYG).HSA<br>also named<br>pSAC35:INSULIN(GYG).F1-N62.HSA | Mature Insulin wherein the C-peptide is replaced by the C-domain of IGF-1 fused upstream of mature HSA and downstream of HSA/kex2 leader. DNA encoding Insulin was codon optimized. | pSAC35            | 356         | 140         | 572         | 895         | 896         | HSA/kex2        |
| 141        | 2256         | pSAC35:VEGF2.T103-R227.HSA  | Amino acids T103 to R227 of VEGF2 fused upstream of mature HSA and downstream of HSA/kex2 leader sequence.  | pSAC35            | 357         | 141         | 573         | 897         | 898         | HSA/kex2        |
| 142        | 2257         | pSAC35:HSA.VEGF2.T103-R227  | Amino acids T103 to R227 of VEGF-2 fused downstream of HSA with HSA/kex2 leader sequence.   | pSAC35            | 358         | 142         | 574         | 899         | 900         | HSA/kex2        |
| 143        | 2271         | pEE12.1:HCHNF25M1-R104.HSA  | Amino acids M1 to R104 of HCHNF25 fused upstream of mature HSA.   | pEE12.1           | 359         | 143         | 575         |             |             | Native HCHNF25  |

**Table 2**

| Fusion No. | Construct ID | Construct Name  | Description  | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence |
|------------|--------------|---|--|-------------------|--------------|--------------|--------------|--------------|--------------|-----------------|
| 144        | 2276         | pSAC35:HSA.INSULIN (GGG)<br>also named:<br>pSAC35:HSA.INSULIN G(GGG).F1-N58 | Mature Insulin wherein the C-peptide is replaced by a synthetic linker fused downstream of HSA. DNA encoding Insulin was codon optimized.  | pSAC35            | 360          | 144          | 576          | 901          | 902          | HSA             |
| 145        | 2278         | pSAC35:insulin(GGG).HSA   | Mature Insulin wherein the C-peptide is replaced by a synthetic linker fused downstream of HSA/kex2 leader and upstream of mature HSA. DNA encoding Insulin was codon optimized.   | pSAC35            | 361          | 145          | 577          | 903          | 904          | HSA/kex2        |
| 146        | 2280         | pC4:HCHNF25.HSA   | HCHNF25 fused upstream of mature HSA.  | pC4               | 362          | 146          | 578          | 905          | 906          | Native HCHNF25  |
| 147        | 2283         | pScCHSA:EPOcoA28-D192.5IN/Q, 65N/Q, 110N/Q EPO                              | Amino acids A28 to D192 of EPO variant (where glycine at amino acid 140 has been replaced with an arginine) are fused upstream of mature HSA and downstream of HSA/kex2 leader sequence. Glycosylation sites at amino acids 51, 65 and 110 are mutated from N to Q residue. DNA encoding EPO is codon optimized. | pScCHSA           | 363          | 147          | 579          | 907          | 908          | HSA/kex2        |

**Table 2**

| Fusion No. | Construct ID | Construct Name                                 | Description   | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence |
|------------|--------------|--|---|-------------------|-------------|-------------|-------------|-------------|-------------|-----------------|
| 148        | 2284         | pScNHSA:EPOcoA28-D192.51N/Q, 65N/Q, 110N/Q EPO | Amino acids A28 to D192 of EPO variant (where glycine at amino acid 140 has been replaced with an arginine) fused downstream of mature HSA and HSA/kex2 leader sequence. Glycosylation sites at amino acids 51, 65 and 110 are mutated from N to Q residue. DNA encoding EPO is codon optimized.            | pScNHSA           | 364         | 148         | 580         | 909         | 910         | HSA/kex2        |
| 149        | 2287         | pSAC35:EPOcoA28-D192.51N/Q,65N/Q,110 N/Q,HSA.  | Amino acids A28 to D192 of EPO variant (where glycine at amino acid 140 has been replaced with an arginine) fused upstream of mature HSA and downstream of HSA/kex2 leader sequence. Glycosylation sites at amino acid 51, 65 and 110 are mutated from N to Q residue. DNA encoding EPO is codon optimized. | pSAC35            | 365         | 149         | 581         | 911         | 912         | HSA/kex2        |
| 150        | 2289         | pSAC35:HSA.EPOcoA28-D192.51N/Q,65N/Q,110 N/Q.  | Amino acids A28 to D192 of EPO variant (where glycine at amino acid 140 has been replaced with an arginine) fused downstream of mature HSA and HSA/kex2 leader sequence. Glycosylation sites at amino acid 51, 65 and 110 are mutated from N to Q residue. DNA encoding EPO is codon optimized.             | pSAC35            | 366         | 150         | 582         | 913         | 914         | HSA/kex2        |



**Table 2**

| Fusion No. | Construct ID | Construct Name                                       | Description   | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence |
|------------|--------------|--|---|-------------------|--------------|--------------|--------------|--------------|--------------|-----------------|
| 151        | 2294         | pC4:EPO.R140G.HSA<br>also named<br>pC4:EPO.R1406.HSA | Amino acids M1-D192 of EPO fused upstream of mature HSA. The EPO sequence included in construct 1997 was used to generate this construct, mutating arginine at EPO amino acid 140 to glycine. This mutated sequence matches the wildtype EPO sequence.    | pC4               | 367          | 151          | 587          | 915          | 916          | Native EPO      |
| 152        | 2295         | pSAC35:humanresistin.<br>K19-P108:HSA                | Amino acids K19 to P108 of Resistin fused upstream of mature HSA and downstream of HSA/kex2 leader sequence.  | pSAC35            | 368          | 152          | 584          | 917          | 918          | HSA/kex2        |
| 153        | 2296         | pSAC35:HSA:humanresistin.K19-P108                    | Amino acids K19 to P108 of Resistin fused downstream of HSA.  | pSAC35            | 369          | 153          | 585          | 919          | 920          | HSA             |
| 154        | 2297         | pSAC35:humanresistin.<br>K19-P108.stop:HSA           | Amino acids K19 to P108 of Resistin fused upstream of mature HSA and downstream of HSA/kex2 leader sequence. Includes two stops at 3' end for termination of translation before the HSA.  | pSAC35            | 370          | 154          | 586          | 921          | 922          | HSA/kex2        |
| 155        | 2298         | pEE12.1:EPO.R140G.HSA                                | Amino acids M1 to D192 of EPO fused upstream of mature HSA. The EPO sequence included in construct 1997 was used to generate this construct, mutating arginine at EPO amino acid 140 to glycine. This mutated sequence matches the wildtype EPO sequence. | pEE12.1           | 371          | 155          | 587          | 923          | 924          | Native EPO      |

**Table 2**

| Fusion No. | Construct ID | Construct Name                    | Description   | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence |
|------------|--------------|-----------------------------------|---|-------------------|--------------|--------------|--------------|--------------|--------------|-----------------|
| 156        | 2300         | pC4:humanresistin.M1-P108:HSA     | Amino acids M1 to P108 of Resistin fused upstream of mature HSA.  | pC4               | 372          | 156          | 588          | 925          | 926          | Native resistin |
| 157        | 2309         | pEE12.1:humanresistin.M1-P108:HSA | Amino acids M1 to P108 of Resistin fused upstream of mature HSA.  | pEE12.1           | 373          | 157          | 589          | 927          |              | Native resistin |
| 158        | 2310         | pc4:EPOco.M1-D192.HSA             | Amino acids M1 to D192 of EPO variant fused upstream of mature HSA. DNA encoding EPO is codon optimized. The EPO sequence included in construct 1997 was used to generate this construct, mutating arginine at EPO amino acid 140 to glycine. This mutated sequence matches the wildtype EPO sequence.      | pC4               | 374          | 158          | 590          | 928          | 929          | Native EPO      |
| 159        | 2311         | pC4:EPO.M1-G27.EPOco.A28-D192.HSA | Amino acids M1 to D192 of EPO fused upstream of mature HSA. DNA encoding only EPO portion is codon optimized. The EPO sequence included in construct 1997 was used to generate this construct, mutating arginine at EPO amino acid 140 to glycine. This mutated sequence matches the wildtype EPO sequence. | pC4               | 375          | 159          | 591          | 930          | 931          | Native EPO      |
| 160        | 2320         | pC4:HCHNF25M1-R104.HSA            | Amino acids M1 to R104 of HCHNF25 fused upstream of mature HSA.   | pC4               | 376          | 160          | 592          | 932          | 933          | Native HCHNF25  |
| 161        | 2325         | pC4:EPO.M1-D192.HSA.Codon opt.    | Amino acids M1 to D192 of EPO fused upstream of mature HSA. DNA encoding EPO is codon optimized.  | pC4               | 377          | 161          | 593          |              |              | Native EPO      |

**Table 2**

| Fusion No. | Construct ID | Construct Name                     | Description   | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence |
|------------|--------------|------------------------------------|---|-------------------|-------------|-------------|-------------|-------------|-------------|-----------------|
| 162        | 2326         | pEE12.1.EPO:M1-D192.HSA.Codon opt. | Amino acids M1 to D192 of EPO fused upstream of mature HSA. DNA encoding EPO is codon optimized.              | pEE12.1           | 378         | 162         | 594         |             |             | Native EPO      |
| 163        | 2328         | pC4:HLDOU18.K23-R429.HSA           | Amino acids K23 to R429 of HLDOU18 fused upstream of mature HSA and downstream of native HSA leader sequence. | pC4               | 379         | 163         | 595         | 934         | 935         | HSA             |
| 164        | 2330         | CK-Beta4-HSA                       | Full length Ckbeta4 fused upstream of mature HSA.   | pSAC35            | 380         | 164         | 596         | 936         | 937         | Native CKβ4     |
| 165        | 2335         | pC4:MPIFsp.ck{b}4D31-M96.HSA       | Amino acids D31 to M96 of Ckbeta4 fused upstream of mature HSA and downstream of MPIF leader sequence.        | pC4               | 381         | 165         | 597         | 938         | 939         | MPIF            |
| 166        | 2336         | pC4:MPIFsp.ck{b}4G35-M96.HSA       | Amino acids G35 to M96 of Ckbeta4 fused upstream of mature HSA and downstream of MPIF leader sequence.        | pC4               | 382         | 166         | 598         | 940         | 941         | MPIF            |
| 167        | 2337         | pC4:MPIFsp.ck{b}4G48-M96.HSA       | Amino acids G48 to M96 of Ckbeta4 fused upstream of mature HSA and downstream of MPIF leader sequence.        | pC4               | 383         | 167         | 599         | 942         | 943         | MPIF            |
| 168        | 2338         | pC4:MPIFsp.ck{b}4A62-M96.HSA       | Amino acids A62 to M96 of Ckbeta4 fused upstream of mature HSA and downstream of MPIF leader sequence.        | pC4               | 384         | 168         | 600         | 944         | 945         | MPIF            |
| 169        | 2340         | pC4:HSA.HLDOU18.K23-R429           | Amino acids K23 to R429 of HLDOU18 fused downstream of HSA.   | pC4               | 385         | 169         | 601         | 946         | 947         | HSA             |
| 170        | 2343         | pSAC35.INV-IFNA2.HSA               | Mature Interferon alpha2 fused upstream of mature HSA and downstream of invertase signal peptide.             | pSAC35            | 386         | 170         | 602         | 948         | 949         | invertase       |

**Table 2**

| Fusion No. | Construct ID | Construct Name                         | Description  | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence |
|------------|--------------|--|--|-------------------|-------------|-------------|-------------|-------------|-------------|-----------------|
| 171        | 2344         | pC4.SpIg.EPO:A28-D192.HSA.Codon opt.   | Amino acids A28 to D192 of EPO fused upstream of mature HSA and downstream of mouse Ig leader sequence. DNA encoding EPO is codon optimized. | pC4               | 387         | 171         | 603         | 950         | 951         | Mouse Ig leader |
| 172        | 2348         | pC4:MPIFsp.ck{b}4G57-M96.HSA           | Amino acids G57 to M96 of Ckbeta4 fused upstream of mature HSA and downstream of MPIF leader sequence.                                       | pC4               | 388         | 172         | 604         | 952         | 953         | MPIF            |
| 173        | 2350         | pC4:MPIFsp.HLDOU18(S320-R429).HSA      | Amino acids S320 to R429 of HLDOU18 fused upstream of mature HSA and downstream of MPIF leader sequence.                                     | pC4               | 389         | 173         | 605         | 954         | 955         | MPIF            |
| 174        | 2351         | pC4:HSA.HLDOU18(S320-R429)             | Amino acids S320 to R429 of HLDOU18 fused downstream of HSA.   | pC4               | 390         | 174         | 606         | 956         | 957         | HSA             |
| 175        | 2355         | pSAC35:MATalpha.d8c kbeta1.G28-N93:HSA | Amino acids G28 to N93 of Ckbeta1 fused upstream of mature HSA and downstream of the yeast mating factor alpha leader sequence.              | pSAC35            | 391         | 175         | 607         | 958         | 959         | MFα-1           |
| 176        | 2359         | pEE12:HLDOU18.K23-R429.HSA             | Amino acids K23 to R429 of HLDOU18 fused upstream of mature HSA and downstream of native HSA leader sequence.                                | pEE12.1           | 392         | 176         | 608         |             |             | HSA             |
| 177        | 2361         | pC4:HRDFD27:HSA                        | HRDFD27 fused upstream of mature HSA.  | pC4               | 393         | 177         | 609         | 960         | 961         | Native HRDFD27  |
| 178        | 2362         | pEE12:HSA.HLDOU18.K23-R429             | Amino acids K23 to R429 of HLDOU18 fused downstream of HSA.  | pEE12.1           | 394         | 178         | 610         |             |             | HSA             |

**Table 2**

| Fusion No. | Construct ID | Construct Name                      | Description  | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence |
|------------|--------------|-------------------------------------|--|-------------------|-------------|-------------|-------------|-------------|-------------|-----------------|
| 179        | 2363         | pC4GCSF.HSA.EPO.A 28-D192           | Amino acids M1 to P204 of GCSF fused upstream of mature HSA which is fused upstream of amino acids A28 to D192 of EPO variant (where amino acid 140 of EPO is mutated from glycine to arginine.)   | pC4               | 395         | 179         | 611         |             |             | Native GCSF     |
| 180        | 2365         | pEE12.1.HCNCA73HS A                 | HCNCA73 is fused upstream of mature HSA.   | pEE12.1           | 396         | 180         | 612         | 962         | 963         | Native HCNCA73  |
| 181        | 2366         | PSAC35.MAF-IFNa2.HSA                | Mature IFNa2 fused upstream of mature HSA and downstream of yeast mating factor alpha leader sequence.   | PSAC35            | 397         | 181         | 613         | 964         | 965         | MFα-1           |
| 182        | 2367         | pEE12.MPIFsp.HLDOU 18.S320-R429.HSA | Amino acids S320 to R429 of HLDOU18 fused upstream of mature HSA and downstream of MPIF leader sequence.   | pEE12.1           | 398         | 182         | 614         | 966         | 967         | MPIF            |
| 183        | 2369         | pC4:HLDOU18.HSA                     | Amino acids M1 to R429 of HLDOU18 fused upstream of mature HSA.  | pC4               | 399         | 183         | 615         | 968         | 969         | Native HLDOU18  |
| 184        | 2370         | pEE12:HLDOU18.HSA                   | Amino acids M1 to R429 of HLDOU18 fused upstream of mature HSA.  | pEE12.1           | 400         | 184         | 616         |             |             | Native HLDOU18  |
| 185        | 2373         | pC4.GCSF.HSA.EPO.A 28-D192.R140G    | Amino acids M1 to P204 of GCSF is fused upstream of mature HSA which is fused upstream of amino acids A28 to D192 of EPO, wherein amino acid 140 is glycine. The EPO sequence included in construct 1997 was used to generate this construct, mutating arginine at EPO amino acid 140 to glycine. This mutated sequence matches the wildtype EPO sequence. | pC4               | 401         | 185         | 617         |             |             | Native GCSF     |

**Table 2**

| Fusion No. | Construct ID | Construct Name                       | Description  | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence        |
|------------|--------------|--------------------------------------|--|-------------------|-------------|-------------|-------------|-------------|-------------|------------------------|
| 186        | 2381         | pC4:HSA-IFNa2(C17-E181)              | Amino acids C17 to E181 of IFNa2 (fragment shown as amino acids C1 to E165 of SEQ ID NO:618) fused downstream of HSA.  | pC4               | 402         | 186         | 618         | 970         | 971         | HSA                    |
| 187        | 2382         | pC4:IFNa2-HSA                        | IFNa2 fused upstream of mature HSA.  | pC4               | 403         | 187         | 619         | 972         | 973         | Native IFNa2 leader    |
| 188        | 2387         | pC4:EPO(G140)-HSA-GCSF.T31-P204      | Amino acids M1-D192 of EPO fused upstream of mature HSA which is fused upstream of amino acids T31 to P204 of GCSF.  | pC4               | 404         | 188         | 620         |             |             | Native EPO             |
| 189        | 2407         | pC4:HWHGZ51.M1-N323.HSA              | Amino acids M1 to N323 of HWHGZ51 fused upstream of mature HSA.  | pC4               | 405         | 189         | 621         | 974         | 975         | Native HWHGZ51         |
| 190        | 2408         | pEE12.1:HWHGZ51.M1-N323.HSA          | Amino acids M1 to N323 of HWHGZ51 fused upstream of mature HSA.  | pEE12.1           | 406         | 190         | 622         | 976         | 977         | Native HWHGZ51         |
| 191        | 2410         | pSAC35INV:IFNa-HSA                   | Mature IFNa2 fused downstream of the invertase signal peptide and upstream of mature HSA.  | pSAC35            | 407         | 191         | 623         | 978         | 979         | invertase              |
| 192        | 2412         | pSAC35:delKEX.d8cbb eta1.G28-N93:HSA | Amino acids G28 to N93 of Ckbeta1 fused downstream of the HSA signal sequence (with the KEX site deleted – last 6 amino acids of the leader) and upstream of mature HSA. | pSAC35            | 408         | 192         | 624         | 980         | 981         | HSA minus the KEX site |

**Table 2**

| Fusion No. | Construct ID | Construct Name  | Description   | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence |
|------------|--------------|---|---|-------------------|-------------|-------------|-------------|-------------|-------------|-----------------|
| 193        | 2414         | pC4:EPO:M1-D192copt.HSA.GCSF.T 31-P204<br>also named:<br>pC4:EPO:M1-D192copt.HAS.GCSF.T 31-P204     | Amino acids M1 to D192 of EPO fused upstream of mature HSA which is fused upstream of amino acids T31 to P204 of GCSF. DNA encoding EPO has been codon optimized.   | pC4               | 409         | 193         | 625         | 982         | 983         | Native EPO      |
| 194        | 2428         | pN4:PTH.S1-Q84/HSA  | Amino acids S1 to Q84 of PTH fused upstream of mature HSA and downstream of the native HSA leader sequence.   | pN4               | 410         | 194         | 626         |             |             | HSA             |
| 195        | 2441         | pEE12:EPO:M1-D192copt.HSA.GCSF.T 31-P204<br>also named:<br>pEE12:EPO:M1-D192copt.HAS.GCSF.T 31-P204 | Amino acids M1 to D192 of EPO fused upstream of mature HSA which is fused upstream of amino acids T31 to P204 of GCSF. DNA encoding EPO has been codon optimized.   | pEE12.1           | 409         | 196         | 628         |             |             | EPO leader      |
| 196        | 2447         | pC4:HSA.humancalcitonin.C1-G33  | Amino acids C98 to G130 of SEQ ID NO:629 fused downstream of HSA.   | pC4               | 413         | 197         | 629         | 986         | 987         | HSA             |
| 197        | 2448         | pSAC35:GLP-1(7-36).HSA  | Amino acids H98 to R127 of preproglucagon (SEQ ID NO:630) (hereinafter this specific domain will be referred to as "GLP-1(7-36)") is fused upstream of mature HSA and downstream of HSA/kex2 leader sequence. | pSAC35            | 414         | 198         | 630         | 988         | 989         | HSA/kex2        |

**Table 2**

| Fusion No. | Construct ID | Construct Name                 | Description   | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence |
|------------|--------------|--------------------------------|---|-------------------|--------------|--------------|--------------|--------------|--------------|-----------------|
| 198        | 2449         | pSAC35:INV.d8CKBI. G28-N93:HSA | Amino acids G28 to N93 of Ckbetal fused downstream of the invertase signal peptide and upstream of mature HSA.  | pSAC35            | 415          | 199          | 631          | 990          | 991          | Invertase       |
| 199        | 2455         | pSAC35:HSA.GLP-1(7-36)         | GLP-1(7-36) is fused downstream of mature HSA and HSA/kex2 leader sequence.   | pSAC35            | 416          | 200          | 632          | 992          | 993          | HSA/kex2        |
| 200        | 2456         | pSAC35:GLP-1(7-36(A8G)).HSA    | Amino acids H98 to R127 of Preproglucagon (SEQ ID NO:633)(also referred to as "GLP-1(7-36)") is mutated at amino acid 99 of SEQ ID NO:633 to replace the alanine with a glycine. This particular GLP-1 mutant will be hereinafter referred to as "GLP-1(7-36(A8G))" and corresponds to the sequence shown in SEQ ID NO:1808. GLP-1(7-36(A8G)) is fused upstream of mature HSA and downstream of HSA/kex2 leader sequence. | pSAC35            | 417          | 201          | 633          | 994          | 995          | HSA/kex2        |
| 201        | 2457         | pSAC35:HSA.GLP-1(7-36(A8G))    | GLP-1(7-36(A8G)) (SEQ ID NO:1808) is fused downstream of mature HSA and HSA/kex2 leader sequence.   | pSAC35            | 418          | 202          | 634          | 996          | 997          | HSA/kex2        |
| 202        | 2469         | pSAC35:HSA.extendin. H48-S86   | Amino acids H48 to S86 of Extendin fused downstream of full length HSA.   | pSAC35            | 419          | 203          | 635          |              |              | HSA             |
| 203        | 2470         | pSAC35:Extendin.H48-S86.HSA    | Amino acids H48 to S86 of Extendin fused upstream of mature HSA and downstream of HSA/kex2 leader sequence.   | pSAC35            | 420          | 204          | 636          |              |              | HSA/kex2        |



**Table 2**

| Fusion No. | Construct ID | Construct Name            | Description   | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence |
|------------|--------------|---------------------------|---|-------------------|--------------|--------------|--------------|--------------|--------------|-----------------|
| 204        | 2473         | pC4.HLDOU18:HSA:S320-R429 | M1-R319 of HLDOU18 (containing the furin site RRKR) followed by residues 'LE' followed by mature HSA followed by 'LE' and amino acids S320 through R429 of HLDOU18 (fragment shown as SEQ ID NO:637). | pC4               | 421          | 205          | 637          | 998          | 999          | Native HLDOU18  |
| 205        | 2474         | pSAC35.MDC.P26-Q93.HSA    | Amino acids P26 to Q93 of MDC fused downstream of the HSA/kex2 leader and upstream of mature HSA.   | pSAC35            | 422          | 206          | 638          | 1000         | 1001         | HSA/kex2        |
| 206        | 2475         | pSAC35.MDC.M26-Q93.HSA    | Amino acids Y27 to Q93 of MDC with an N-terminal methionine, fused downstream of the HSA/kex2 leader and upstream of mature HSA.  | pSAC35            | 423          | 207          | 639          | 1002         | 1003         | HSA/kex2        |
| 207        | 2476         | pSAC35.MDC.Y27-Q93.HSA    | Amino acids Y27 to Q93 of MDC fused downstream of the HSA/kex2 leader and upstream of mature HSA.   | pSAC35            | 424          | 208          | 640          | 1004         | 1005         | HSA/kex2        |
| 208        | 2477         | pSAC35.MDC.M27-Q93.HSA    | Amino acids G28 to Q93 of MDC with an N-terminal methionine, fused downstream of the HSA/kex2 leader and upstream of mature HSA.  | pSAC35            | 425          | 209          | 641          | 1006         | 1007         | HSA/kex2        |
| 209        | 2489         | pSAC35:HSA.C17.A20-R136   | Amino acids A20 to R136 of C17 fused downstream of mature HSA with HSA/kex2 leader sequence.  | pSAC35            | 426          | 210          | 642          | 1008         | 1009         | HSA/kex2        |
| 210        | 2490         | pSAC35:C17.A20-R136.HSA   | Amino acids A20 to R136 of C17 fused downstream of the HSA/kex2 leader and upstream of mature HSA.  | pSAC35            | 427          | 211          | 643          | 1010         | 1011         | HSA/kex2        |

**Table 2**

| Fusion No. | Construct ID | Construct Name                  | Description  | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence    |
|------------|--------------|---------------------------------|--|-------------------|--------------|--------------|--------------|--------------|--------------|--------------------|
| 211        | 2492         | pC4:IFNb(deltaM22).HSA          | Mutant full length INFbeta fused upstream of mature HSA. First residue of native, mature INFbeta (M22) has been deleted. | pC4               | 428          | 212          | 644          |              |              | Native INFβ leader |
| 212        | 2498         | pC4:HSA.KGF2D60.G96-S208        | Amino acids G96 to S208 of KGF-2 fused downstream of HSA.  | pC4               | 429          | 213          | 645          | 1012         | 1013         | HSA                |
| 213        | 2499         | pC4:KGF2D60.G96-S208:HSA        | Amino acids G96 to S208 of KGF2 fused upstream of mature HSA and downstream of the HSA signal peptide.                   | pC4               | 430          | 214          | 646          | 1014         | 1015         | HSA                |
| 214        | 2501         | pSAC35:scFvI006D08.HSA          | BLyS antibody fused upstream of mature HSA and downstream of HSA/kex2 signal peptide.                                    | pSAC35            | 431          | 215          | 647          | 1016         | 1017         | HSA/kex2           |
| 215        | 2502         | pSAC35:scFvI050B11.HSA          | BLyS antibody fused upstream of mature HSA and downstream of HSA/kex2 leader sequence.                                   | pSAC35            | 432          | 216          | 648          | 1018         | 1019         | HSA/kex2           |
| 216        | 2513         | pC4:HSA.salmoncalcitonin.C1-G33 | C1 through G33 of salmon calcitonin fused downstream of full length HSA.   | pC4               | 1513         | 1345         | 1681         | 1854         | 1855         | HSA                |
| 217        | 2515         | pC4:HDPBQ71.M1-N565.HSA         | M1 through N565 of HDPBQ71 fused upstream of mature HSA  | pC4               | 1514         | 1346         | 1682         | 1856         | 1857         | Native HDPBQ71     |
| 218        | 2529         | pC4:TR1.M1-K194.HSA             | Amino acids M1 to K194 of TR1 (including native signal sequence) fused upstream of mature HSA.                           | pC4               | 1223         | 1208         | 1238         | 1253         | 1254         | Native TR1         |
| 219        | 2530         | pC4:TR1.M1-Q193.HSA             | Amino acids M1 to Q193 of TR1 (including native signal sequence) fused upstream of mature HSA.                           | pC4               | 1224         | 1209         | 1239         | 1255         | 1256         | Native TR1         |

**Table 2**

| Fusion No. | Construct ID | Construct Name                  | Description  | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence |
|------------|--------------|---------------------------------|--|-------------------|-------------|-------------|-------------|-------------|-------------|-----------------|
| 220        | 2531         | pC4:TR1.M1-E203.HSA             | Amino acids M1 to E203 of TR1 (including native signal sequence) fused upstream of mature HSA. | pC4               | 1225        | 1210        | 1240        | 1257        | 1258        | Native TR1      |
| 221        | 2532         | pC4:TR1.M1-Q339.HSA             | Amino acids M1 to Q339 of TR1 (including native signal sequence) fused upstream of mature HSA. | pC4               | 1226        | 1211        | 1241        | 1259        | 1260        | Native TR1      |
| 222        | 2545         | pEE12.1:HDPBQ71.M1-N565.HSA     | M1 through N565 of HDPBQ71 fused upstream of mature HSA  | pEE12.1           | 1515        | 1347        | 1683        |             |             | Native HDPBQ71  |
| 223        | 2552         | pSAC35:KGF2delta33.S69-S208.HSA | Amino acids S69 through S208 of KGF2 fused upstream of HSA.                                    | pScCHSA           | 1516        | 1348        | 1684        | 1858        | 1859        | HSA/kex2        |
| 224        | 2553         | pSAC35:HSA.KGF2delta33.S69-S208 | HSA/kex2 signal peptide followed by HSA peptide followed by amino acids S69 to S208 of KGF2.   | pScNHSA           | 1517        | 1349        | 1685        | 1860        | 1861        | HSA/kex2        |
| 225        | 2555         | pEE12.1:TR1.M1-Q193.HSA         | Amino acids M1 to Q193 of TR1 (including native signal sequence) fused upstream of mature HSA. | pEE12.1           | 1227        | 1212        | 1242        |             |             | Native TR1      |
| 226        | 2556         | pEE12.1:TR1.M1-K194.HSA         | Amino acids M1 to K194 of TR1 (including native signal sequence) fused upstream of mature HSA. | pEE12.1           | 1228        | 1213        | 1243        |             |             | Native TR1      |
| 227        | 2557         | pEE12.1:TR1.M1-E203.HSA         | Amino acids M1 to E203 of TR1 (including native signal sequence) fused upstream of mature HSA. | pEE12.1           | 1229        | 1214        | 1244        |             |             | Native TR1      |
| 228        | 2558         | pEE12.1:TR1.M1-Q339.HSA         | Amino acids M1 to Q339 of TR1 (including native signal sequence) fused upstream of mature HSA. | pEE12.1           | 1230        | 1215        | 1245        |             |             | Native TR1      |

**Table 2**

| Fusion No. | Construct ID | Construct Name                       | Description  | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence              |
|------------|--------------|--------------------------------------|--|-------------------|--------------|--------------|--------------|--------------|--------------|------------------------------|
| 229        | 2571         | pC4.OSCAR.R232.HSA                   | M1-R232 of OSCAR fused upstream of mature HSA.   | pC4               | 1518         | 1350         | 1686         | 1862         | 1863         | Native OSCAR receptor leader |
| 230        | 2580         | pC4.IFNb(deltaM22,C3 8S).HSA         | IFNb fused upstream of mature HSA. The IFNb used in this fusion lacks the first residue of the mature form of IFNb, which corresponds to M22 of SEQ ID NO:1687. Also amino acid 38 of SEQ ID NO:1687 has been mutated from Cys to Ser. | pC4               | 1519         | 1351         | 1687         |              |              | Native IFN $\beta$           |
| 231        | 2584         | pC4:MPIFsp.KGF2delta 28.A63-S208.HSA | MPIF signal sequence followed by A63 through S208 of KGF2 followed by mature HSA.  | pC4               | 1520         | 1352         | 1688         | 1864         | 1865         | MPIF                         |
| 232        | 2603         | pC4:HSA(A14)-EPO(A28-D192.G140)      | Modified HSA A14 leader fused upstream of mature HSA which is fused upstream of A28 through D192 of EPO. Amino acid 140 of EPO is a 'G'.   | pC4               | 1521         | 1353         | 1689         |              |              | Modified HSA (A14)           |
| 233        | 2604         | pC4:HSA(S14)-EPO(A28-D192.G140)      | Modified HSA S14 leader fused upstream of mature HSA which is fused upstream of A28 through D192 of EPO. Amino acid 140 of EPO is a 'G'.   | pC4               | 1522         | 1354         | 1690         |              |              | Modified HSA (S14)           |
| 234        | 2605         | pC4:HSA(G14)-EPO(A28-D192.G140)      | Modified HSA G14 leader fused upstream of mature HSA which is fused upstream of A28 through D192 of EPO. Amino acid 140 of EPO is a 'G'.   | pC4               | 1523         | 1355         | 1691         |              |              | Modified HSA (G14)           |

**Table 2**

| Fusion No. | Construct ID | Construct Name                             | Description  | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence           |
|------------|--------------|--|--|-------------------|-------------|-------------|-------------|-------------|-------------|---------------------------|
| 235        | 2606         | pC4:HSA#64.KGF2D28.A63-S208                | A63 through S208 of KGF2 fused downstream of mature HSA and the modified #64 leader sequence.          | pC4               | 1524        | 1356        | 1692        | 1866        | 1867        | Modified HSA #64          |
| 236        | 2607         | pC4:HSA#65.KGF2D28.A63-S208                | A63 through S208 of KGF2 downstream of mature HSA and the modified #65 leader sequence.                | pC4               | 1525        | 1357        | 1693        | 1868        | 1869        | Modified HSA #65          |
| 237        | 2608         | pC4:HSA#66.KGF2D28.A63-S208                | A63 through S208 of KGF2 fused downstream of mature HSA and the modified #66 leader sequence.          | pC4               | 1526        | 1358        | 1694        | 1870        | 1871        | Modified HSA #66          |
| 238        | 2623         | pC4:(AGVSG,14-18)HSA.HLDOU18.K2 3-R429     | A modified HSA A14 leader followed by mature HSA and amino acids K23 through R429 of HLDOU18.          | pC4               | 1527        | 1359        | 1695        |             |             | Modified HSA (A14) leader |
| 239        | 2624         | pC4:(SGVSG,14-18)HSA.HLDOU18.K2 3-R429     | Modified HSA S14 leader followed by mature HSA and amino acids K23 to R429 of HLDOU18.                 | pC4               | 1528        | 1360        | 1696        |             |             | Modified HSA (S14) leader |
| 240        | 2625         | pC4:(GGVSG,14-18)HSA.HLDOU18.K2 3-R429     | A modified HSA G14 leader sequence followed by mature HSA and amino acids K23 through R429 of HLDOU18. | pC4               | 1529        | 1361        | 1697        |             |             | Modified HSA (G14) leader |
| 241        | 2630         | pC4:HSA.KGF2D28.A63-S208#2                 | Amino acids A63 to S208 of KGF-2 fused to the C-terminus of HSA.                                       | pC4               | 1530        | 1362        | 1698        | 1872        | 1873        | HSA                       |
| 242        | 2631         | pEE12.1:(AGVSG,14-18)HSA.HLDOU18.K2 3-R429 | A modified HSA A14 leader sequence followed by mature HSA and amino acids K23 through R429 of HLDOU18. | pEE12.1           | 1531        | 1363        | 1699        |             |             | Modified HSA (A14) leader |
| 243        | 2632         | pEE12.1:(SGVSG,14-18)HSA.HLDOU18.K2 3-R429 | Modified HSA S14 leader followed by mature HSA and amino acids K23 to R429 of HLDOU18.                 | pEE12.1           | 1532        | 1364        | 1700        |             |             | Modified HSA (S14) leader |

**Table 2**

| Fusion No. | Construct ID | Construct Name                             | Description   | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence           |
|------------|--------------|--|---|-------------------|--------------|--------------|--------------|--------------|--------------|---------------------------|
| 244        | 2633         | pEE12.1:(GGVSG,14-18)HSA.HLDOU18.K2 3-R429 | A modified HSA G14 leader sequence followed by mature HSA and amino acids K23 through R429 of HLDOU18.        | pEE12.1           | 1533         | 1365         | 1701         |              |              | Modified HSA (G14) leader |
| 245        | 2637         | pSAC35:HSA.GCSF.T3 1-P207                  | HSA/kex2 leader fused upstream of mature HSA followed by T31 through P207 of GCSF (SEQ ID NO:1702).           | pScNHSA           | 1534         | 1366         | 1702         | 1874         | 1875         | HSA/kex2                  |
| 246        | 2638         | pPPC007:116A01.HSA                         | scFv I116A01 with C-terminal HSA fusion, where the mature form of HSA lacks the first 8 amino acids.          | pPPC007           | 1535         | 1367         | 1703         | 1876         | 1877         | scFvI006A 01              |
| 247        | 2647         | pSAC35:T7.HSA.                             | The T7 peptide (SEQ ID NO: 1704) of Tumstatin was fused with a C-terminal HSA and N terminal HSA/kex2 leader. | pScCHSA           | 1536         | 1368         | 1704         | 1878         | 1879         | HSA/kex2                  |
| 248        | 2648         | pSAC35:T8.HSA                              | The T8 peptide (SEQ ID NO: 1705) of Tumstatin is fused upstream to mature HSA and downstream from HSA/kex2.   | pScCHSA           | 1537         | 1369         | 1705         | 1880         | 1881         | HSA/kex2                  |
| 249        | 2649         | pSAC35:HSA.T7                              | The T7 peptide (SEQ ID NO: 1706) of Tumstatin was fused with a N-terminal HSA/kex2 signal sequence.           | pScNHSA           | 1538         | 1370         | 1706         | 1882         | 1883         | HSA/kex2                  |
| 250        | 2650         | pSAC35:HSA.T8                              | The T8 peptide (SEQ ID NO: 1767) of Tumstatin is fused downstream to HSA/kex2 signal sequence and mature HSA. | pScNHSA           | 1539         | 1371         | 1707         | 1884         | 1885         | HSA/kex2                  |

**Table 2**

| Fusion No. | Construct ID | Construct Name                 | Description  | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence |
|------------|--------------|--------------------------------|--|-------------------|-------------|-------------|-------------|-------------|-------------|-----------------|
| 251        | 2656         | pSac35:Insulin(KR.GG.G.KR).HSA | Synthetic gene coding for a single-chain insulin with HSA at C-terminus. Contains a modified loop for processing resulting in correctly disulfide bonded insulin coupled to HSA. | pScCHSA           | 1540        | 1372        | 1708        | 1886        | 1887        | HSA/kex2        |
| 252        | 2667         | pSAC35:HSA.T1249               | T1249 fused downstream of full length HSA  | pSAC35            | 1178        | 1179        | 1180        | 1181        | 1182        | HSA             |
| 253        | 2668         | pSac35:HSA.Insulin(KR.GGG.KR)  | Synthetic gene coding for insulin with FL HSA at N-terminus. Contains a modified loop for processing resulting in correctly disulfide bonded insulin coupled to HSA.             | pScNHSA           | 1541        | 1373        | 1709        | 1888        | 1889        | HSA             |
| 254        | 2669         | pSac35:Insulin(GGG.K.K).HSA    | Synthetic gene coding for a single-chain insulin with HSA at C-terminus. Contains a modified loop.   | pScCHSA           | 1542        | 1374        | 1710        | 1890        | 1891        | HSA/kex2        |
| 255        | 2670         | pSAC35:T1249.HSA               | T1249 fused downstream of HSA/kex2 leader and upstream of mature HSA.  | pSAC35            | 1183        | 1179        | 1180        | 1184        | 1185        | HSA/kex2        |
| 256        | 2671         | pSac35:HSA.Insulin(GG.G.KK)    | Synthetic gene coding for a single-chain insulin with HSA at N-terminus. Contains a modified loop for greater stability.   | pScNHSA           | 1543        | 1375        | 1711        | 1892        | 1893        | HSA             |
| 257        | 2672         | pSAC35:HSA.T20                 | Amino terminus of T20 (codon optimized) fused downstream of full length HSA  | pSAC35            | 1186        | 1187        | 1188        | 1189        | 1190        | HSA             |
| 258        | 2673         | pSAC35:T20.HSA                 | Amino terminus of T20 (codon optimized) fused downstream of HSA/kex2 leader and upstream of mature HSA.  | pSAC35            | 1191        | 1187        | 1188        | 1192        | 1193        | HSA/kex2        |
| 259        | 2700         | pSAC35:HSA.GCSF.T3 1-R199      | C-terminal deletion of GCSF fused downstream of mature HSA.  | pSAC35            | 1544        | 1376        | 1712        | 1894        | 1895        | HSA/kex2        |

**Table 2**

| Fusion No. | Construct ID | Construct Name                   | Description   | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence           |
|------------|--------------|----------------------------------|---|-------------------|-------------|-------------|-------------|-------------|-------------|---------------------------|
| 260        | 2701         | pSAC35:HSA.GCSF.T3 1-H200        | C-terminal deletion of GCSF fused downstream of mature HSA.   | pScNHSA           | 1545        | 1377        | 1713        | 1896        | 1897        | HSA/kex2                  |
| 261        | 2702         | pSAC35:HSA.GCSF.T3 1-L201        | HSA/kex2 leader followed by mature HSA and amino acids T31-L201 of GCSF (corresponding to amino acids T1 to L171 of SEQ ID NO:1196).  | pSAC35            | 1194        | 1195        | 1196        | 1197        | 1198        | HSA/kex2                  |
| 262        | 2703         | pSAC35:HSA.GCSF.A3 6-P204        | HSA/kex2 leader followed by mature HSA and amino acids A36-P204 of GCSF.  | pScNHSA           | 1546        | 1378        | 1714        | 1898        | 1899        | HSA/kex2                  |
| 263        | 2714         | pC4:HSASP.PTH34(2)/HSA           | PTH34 double tandem repeats fused downstream of HSA leader (with the KEX site deleted – last 6 amino acids of the leader) and upstream of mature HSA.                                 | pC4               | 1199        | 1200        | 1201        | 1202        | 1203        | HSA leader minus Kex site |
| 264        | 2724         | pSAC35.sCNTF.HSA                 | HSA/Kex2 fused to CNTF, and then fused to mature HSA.   | pSAC35            | 1547        | 1379        | 1715        | 1900        | 1901        | HSA/kex2                  |
| 265        | 2725         | pSAC35:HSA.sCNTF                 | HSA/Kex2 fused to mature HSA and then to CNTF   | pSAC35            | 1548        | 1380        | 1716        | 1902        | 1903        | HSA/kex2                  |
| 266        | 2726         | pSac35.INV.GYGinsulin.HSA        | Synthetic gene coding for a single-chain insulin with HSA at C-terminus. The signal peptide of invertase is used for this construct.  | pSAC35            | 1549        | 1381        | 1717        | 1904        | 1905        | Invertase                 |
| 267        | 2727         | pSac35.INV.GYGinsulin(delF1).HSA | Synthetic gene coding for a single-chain insulin with HSA at C-terminus. Construct uses the invertase signal peptide and is lacking the first amino acid (F) of mature human insulin. | pSAC35            | 1550        | 1382        | 1718        | 1906        | 1907        | invertase                 |



**Table 2**

| Fusion No. | Construct ID | Construct Name                             | Description   | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence           |
|------------|--------------|--|---|-------------------|--------------|--------------|--------------|--------------|--------------|---------------------------|
| 268        | 2749         | pEE12.1.OSCAR.R232. HSA                    | Amino acids M1 through R232 of OSCAR fused upstream of mature HSA.  | pEE12.1           | 1551         | 1383         | 1719         | 1908         | 1909         | Native OSCAR leader       |
| 269        | 2784         | pSAC35:Insulin(GYG)-HSA codon optimized    | Synthetic gene coding for a single-chain insulin with HSA at C-terminus.  | pSAC35            | 1552         | 1384         | 1720         | 1910         | 1911         | invertase                 |
| 270        | 2789         | pSAC35:Insulin(GGG). HSA (codon optimized) | Synthetic gene coding for a single-chain insulin with HSA at C-terminus.  | pSAC35            | 1553         | 1385         | 1721         | 1912         | 1913         | invertase                 |
| 271        | 2791         | pEE12.1:HSAsp.PTH34 (2X) HSA               | Parathyroid hormone is fused in tandem and upstream of mature HSA and downstream from HSA signal peptide (with the KEX site deleted – last 6 amino acids of the leader) | pEE12.1           | 1554         | 1386         | 1722         |              |              | HSA leader minus Kex site |
| 272        | 2795         | pC4:HSA(A14)-IFNb.M22-N187                 | The mature form of IFNb is fused to the C-terminus of HSA, which contains an modified signal peptide, designed to improve processing and homogeneity.                   | pC4               | 1555         | 1387         | 1723         |              |              | Modified HSA (A14)        |
| 273        | 2796         | pC4:HSA(S14)-IFNb.M22-N187                 | The mature form of IFNb is fused to the C-terminus of HSA, which contains a modified signal peptide, designed to improve processing and homogeneity.                    | pC4               | 1556         | 1388         | 1724         |              |              | Modified HSA (S14)        |
| 274        | 2797         | pC4:HSA(G14)-IFNb.M22-N187                 | The mature form of IFNb is fused to the C-terminus of HSA, which contains an modified signal peptide.   | pC4               | 1557         | 1389         | 1725         |              |              | Modified HSA (G14)        |
| 275        | 2798         | pSAC35:Somatostatin(S14).HSA               | A 14 amino acid peptide of Somatostatin fused downstream of HSA/kex2 leader and upstream of mature HSA.   | pScCHSA           | 1558         | 1390         | 1726         | 1914         | 1915         | HSA/kex2                  |

**Table 2**

| Fusion No. | Construct ID | Construct Name                     | Description  | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence                           |
|------------|--------------|------------------------------------|--|-------------------|--------------|--------------|--------------|--------------|--------------|---|
| 276        | 2802         | pSAC35:GLP-1(7-36(A8G)).IP2.HSA    | GLP-1(7-36(A8G)) (SEQ ID NO:1808) is fused downstream from the HSA/kex2 leader sequence and upstream from the intervening peptide-2 of proglucagon peptide and upstream from mature HSA. | pScNHSA           | 1559         | 1391         | 1727         |              |              | HSA/kex2                                  |
| 277        | 2803         | pSAC35:GLP-1(7-36(A8G))x2.HSA      | GLP-1(7-36(A8G)) (SEQ ID NO:1808) is tandemly repeated and fused downstream of the HSA/kex2 signal sequence, and upstream of mature HSA.   | pScCHSA           | 1231         | 1216         | 1246         | 1261         | 1262         | HSA/kex2                                  |
| 278        | 2804         | pSAC35:coGLP-1(7-36(A8G))x2.HSA    | GLP-1(7-36(A8G)) (SEQ ID NO:1808) is tandemly repeated and fused downstream of the HSA/kex2 signal sequence, and upstream of mature HSA.   | pScCHSA           | 1232         | 1217         | 1247         | 1263         | 1264         | HSA/kex2                                  |
| 279        | 2806         | pC4:HSA#65.salmonal citonin.C1-G33 | Modified HSA leader #65 followed by mature HSA and C1-G33 of salmon calcitonin.  | pC4               | 1560         | 1392         | 1728         | 1916         | 1917         | Modified HSA #65                          |
| 280        | 2821         | pSac35.delKex2.Insulin(GYG).HSA    | Synthetic gene coding for a single-chain insulin with HSA at C-terminus. The kex2 site has been deleted from the HSA/KEX2 signal peptide.  | pScCHSA           | 1561         | 1393         | 1729         |              |              | Modified HSA/kex2, lacking the Kex2 site. |
| 281        | 2822         | pSac35.alphaMF.Insulin(GYG).HSA    | Synthetic gene coding for a single-chain insulin with HSA at C-terminus. The signal peptide of alpha mating factor (MF $\alpha$ -1) is used for this construct.                          | pSAC35            | 1562         | 1394         | 1730         | 1920         | 1921         | MF $\alpha$ -1                            |
| 282        | 2825         | pSAC35:HSA.Somatostatin(S14)       | 14 amino acid peptide of Somatostatin was fused downstream of HSA/kex2 leader and mature HSA.  | pScNHSA           | 1563         | 1395         | 1731         | 1922         | 1923         | HSA/kex2                                  |

**Table 2**

| Fusion No. | Construct ID | Construct Name                             | Description   | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence |
|------------|--------------|--|---|-------------------|-------------|-------------|-------------|-------------|-------------|-----------------|
| 283        | 2830         | pSAC35:S28.HSA                             | 28 amino acids of somatostatin fused downstream of HSA/kex2 leader and upstream of mature HSA.                                      | pScCHSA           | 1564        | 1396        | 1732        | 1924        | 1925        | HSA/kex2        |
| 284        | 2831         | pSAC35:HSA.S28                             | 28 amino acids of somatostatin fused downstream of HSA/kex2 leader and mature HSA.  | pScNHSA           | 1565        | 1397        | 1733        | 1926        | 1927        | HSA/kex2        |
| 285        | 2832         | pSAC35:Insulin.HSA (yeast codon optimized) | Long-acting insulin peptide fused upstream of mature HSA.   | pScCHSA           | 1566        | 1398        | 1734        | 1928        | 1929        | invertase       |
| 286        | 2837         | pSAC35:CKB1.K21-N93:HSA                    | K21-N93 of CKB1 (fragment shown as K2 to N74 of SEQ ID NO:1735) fused downstream of the HSA/kex2 leader and upstream of mature HSA. | pScCHSA           | 1567        | 1399        | 1735        | 1930        | 1931        | HSA/kex2        |
| 287        | 2838         | pSAC35:CKB1.T22-N93:HSA                    | T22-N93 of CKB1 (fragment shown as T3 to N74 of SEQ ID NO:1736) fused downstream of the HSA/kex2 leader and upstream of mature HSA. | pScCHSA           | 1568        | 1400        | 1736        | 1932        | 1933        | HSA/kex2        |
| 288        | 2839         | pSAC35:CKB1.E23-N93:HSA                    | E23-N93 of CKB1 (fragment shown as E4 to N74 of SEQ ID NO:1737) fused downstream of the HSA/kex2 leader and upstream of mature HSA. | pScCHSA           | 1569        | 1401        | 1737        | 1934        | 1935        | HSA/kex2        |
| 289        | 2840         | pSAC35:CKB1.S24-N93:HSA                    | S24-N93 of CKB1 (fragment shown as S5 to N74 of SEQ ID NO:1738) fused downstream of the HSA/kex2 leader and upstream of mature HSA. | pScCHSA           | 1570        | 1402        | 1738        | 1936        | 1937        | HSA/kex2        |

**Table 2**

| Fusion No. | Construct ID | Construct Name                       | Description   | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence |
|------------|--------------|--------------------------------------|---|-------------------|-------------|-------------|-------------|-------------|-------------|-----------------|
| 290        | 2841         | pSAC35:CKB1.S25-N93:HSA              | S25-N93 of CKB1 (fragment shown as S6 to N74 of SEQ ID NO:1739) fused downstream of the HSA/kex2 leader and upstream of mature HSA.     | pScCHSA           | 1571        | 1403        | 1739        | 1938        | 1939        | HSA/kex2        |
| 291        | 2842         | pSAC35:CKB1.S26-N93:HSA              | S26-N93 of CKB1 (fragment shown as S7 to N74 of SEQ ID NO:1740) fused downstream of the HSA/kex2 leader and upstream of mature HSA.     | pScCHSA           | 1572        | 1404        | 1740        | 1940        | 1941        | HSA/kex2        |
| 292        | 2843         | pSAC35:CKB1.R27-N93:HSA              | R27-N93 of CKB1 (fragment shown as R8 to N74 of SEQ ID NO:1741) fused downstream of the HSA/kex2 leader and upstream of mature HSA.     | pScCHSA           | 1573        | 1405        | 1741        | 1942        | 1943        | HSA/kex2        |
| 293        | 2844         | pSAC35:CKB1.P29-N93:HSA              | P29-N93 of CKB1 (fragment shown as P10 to N74 of SEQ ID NO:1742) fused downstream of the HSA/kex2 leader and upstream of mature HSA.    | pScCHSA           | 1574        | 1406        | 1742        | 1944        | 1945        | HSA/kex2        |
| 294        | 2845         | pSAC35:CKB1.Y30-N93:HSA              | Y30-N93 of CKB1 (fragment shown as Y11 to N74 of SEQ ID NO:1743) fused downstream of the HSA/kex2 leader and upstream of mature HSA.    | pScCHSA           | 1575        | 1407        | 1743        | 1946        | 1947        | HSA/kex2        |
| 295        | 2849         | pC4.MPIFsp.CKB1.G28-N93:HSA          | G28-N93 of CKB1 (fragment shown as G9 to N74 of SEQ ID NO:1744) fused downstream of the MPIF signal peptide and upstream of mature HSA. | pC4               | 1576        | 1408        | 1744        | 1948        | 1949        | MPIF            |
| 296        | 2872         | pSAC35:HSA.IFNαA(C1-Q91)/D(L93-E166) | This construct contains a hybrid form of IFNαA and IFNαD fused downstream of mature HSA.  | pSAC35            | 1309        | 1310        | 1311        | 1312        | 1313        | HSA/kex2        |

**Table 2**

| Fusion No. | Construct ID | Construct Name                                    | Description  | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence  |
|------------|--------------|---|--|-------------------|--------------|--------------|--------------|--------------|--------------|------------------|
| 297        | 2873         | pSAC35:HSA.IFNαA(C1-Q91)/ B(L93-E166)             | This construct contains a hybrid form of IFNαA and IFNαB fused downstream of mature HSA.                       | pSAC35            | 1314         | 1315         | 1316         | 1317         | 1318         | HSA/kex2         |
| 298        | 2874         | pSAC35:HSA.IFNαA(C1-Q91)/ F(L93-E166)             | This construct contains a hybrid form of IFNαA and IFNαF fused downstream of mature HSA.                       | pSAC35            | 1319         | 1320         | 1321         | 1322         | 1323         | HSA/kex2         |
| 299        | 2875         | pSAC35:HSA.IFNαA(C1Q-62)/D(Q64-E166)              | This construct contains a hybrid form of IFNαA and IFNαD fused downstream of mature HSA.                       | pSAC35            | 1324         | 1325         | 1326         | 1327         | 1328         | HSA/kex2         |
| 300        | 2876         | pSAC35:HSA.IFNαA(C1-Q91)/ D(L93-E166); R23K,A113V | This construct contains a hybrid form of IFNαA and IFNαD fused downstream of mature HSA.                       | pSAC35            | 1329         | 1330         | 1331         | 1332         | 1333         | HSA/kex2         |
| 301        | 2877         | pSAC35:KT.Insulin.HSA                             | Killer toxin signal peptide fused to synthetic gene coding for a single-chain insulin with C-terminal HSA      | pScCHSA           | 1577         | 1409         | 1745         | 1950         | 1951         | Killer toxin     |
| 302        | 2878         | pSAC35:AP.Insulin.HSA                             | Acid phosphatase signal peptide fused to synthetic gene coding for a single-chain insulin with C-terminal HSA. | pSAC35            | 1578         | 1410         | 1746         |              |              | Acid phosphatase |
| 303        | 2882         | pSac35.alpha.MFprepro.I nsulin(GYG).HSA           | MFα-1 prepro signal followed by GYG insulin followed by mature HSA.  | pSAC35            | 1579         | 1411         | 1747         |              |              | MFα-1            |
| 304        | 2885         | pSac35.alpha.MFpreproE A.Insulin(GYG).HSA         | Yeast MFα-1 prepro signal followed by GYG insulin followed by mature HSA.                                      | pSAC35            | 1580         | 1412         | 1748         |              |              | Yeast MFα-1      |
| 305        | 2886         | pSAC35:HSA.GCSF.P40-P204                          | HSA/kex2 signal peptide followed by mature HSA followed by GCSF (P40-P204).                                    | pSAC35            | 1581         | 1413         | 1749         | 1952         | 1953         | HSA/kex2         |

**Table 2**

| Fusion No. | Construct ID | Construct Name                | Description  | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence |
|------------|--------------|-------------------------------|--|-------------------|--------------|--------------|--------------|--------------|--------------|-----------------|
| 306        | 2887         | pSAC35:HSA.GCSF.P40-L201      | HSA/kex2 signal peptide followed by mature HSA followed by GCSF (P40-L201).  | pSAC35            | 1582         | 1414         | 1750         | 1954         | 1955         | HSA/kex2        |
| 307        | 2888         | pSAC35:HSA.GCSF.Q41-L201      | HSA/kex2 signal peptide followed by mature HSA followed by GCSF (Q41-L201).  | pSAC35            | 1583         | 1415         | 1751         | 1956         | 1957         | HSA/kex2        |
| 308        | 2889         | pSAC35:HSA.GCSF.Q41-P204      | HSA/kex2 signal peptide followed by mature HSA followed by GCSF (Q41-P204).  | pSAC35            | 1584         | 1416         | 1752         | 1958         | 1959         | HSA/kex2        |
| 309        | 2890         | pC4.HSA.GCSF.T31-P204         | HSA/kex2 signal peptide followed by mature HSA followed by GCSF (T31-P204).  | pC4               | 1585         | 1417         | 1753         | 1960         | 1961         | HSA/kex2        |
| 310        | 2891         | pGAP.alphaMF.Insulin(GYG).HSA | Synthetic gene coding for a single-chain insulin with HSA at C-terminus. The signal peptide of HSA/kex2 is used for this construct.  | pYPGaf            | 1586         | 1418         | 1754         | 1962         | 1963         | HSA/kex2        |
| 311        | 2897         | pGAP.Insulin(KR.GGG.KR).HSA   | Long-acting insulin analog using a synthetic gene coding for a single-chain insulin with HSA at C-terminus. Contains a modified loop for processing resulting in correctly disulfide bonded insulin coupled to HSA | pYPGaf            | 1587         | 1419         | 1755         | 1964         | 1965         | HSA/kex2        |
| 312        | 2900         | pSAC:GLP-1(7-36)x2.HSA        | GLP-1(7-36) is tandemly repeated and then fused downstream of the HSA/kex2 signal sequence and upstream of mature HSA.   | pScCHSA           | 1233         | 1218         | 1248         | 1265         | 1266         | HSA/kex2        |

**Table 2**

| Fusion No. | Construct ID | Construct Name                     | Description  | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence |
|------------|--------------|------------------------------------|--|-------------------|--------------|--------------|--------------|--------------|--------------|-----------------|
| 313        | 2901         | pSAC35:IL22.A18-P202.HSA           | Amino acids A18-P202 of IL22 fused downstream of HSA/kex2 leader and upstream of mature HSA.   | pSAC35            | 1588         | 1420         | 1756         | 1966         | 1967         | HSA/kex2        |
| 314        | 2902         | pSAC35: Somatostatin(S14(A-G)).HSA | A 14 amino acid peptide of Somatostatin, an inhibitor of growth hormone, synthesized as a C-terminal HSA fusion. Somatostatin has an alanine to glycine change at amino acid 1 of SEQ ID NO:1757.                                    | pScCHSA           | 1589         | 1421         | 1757         | 1968         | 1969         | HSA/kex2        |
| 315        | 2903         | pSAC35:HSA.A18-P202.IL22           | Amino acids A18-P202 of IL22 fused downstream of full length HSA.  | pSAC35            | 1590         | 1422         | 1758         | 1970         | 1971         | HSA             |
| 316        | 2904         | pSAC35:GLP-1(9-36).HSA             | Amino acids E100 to R127 of preproglucagon (SEQ ID NO:1249) (hereinafter, this particular mutant is referred to as GLP-1(9-36)) is fused downstream from the HSA/kex2 signal sequence and upstream from GLP-1(7-36), and mature HSA. | pScCHSA           | 1234         | 1219         | 1249         | 1267         | 1268         | HSA/kex2        |
| 317        | 2908         | pSAC35:HSA.HCE1P8 <sub>0</sub>     | Mature HSA fused downstream of the HSA/kex2 leader and upstream of HCE1P80.  | pSAC35            | 1591         | 1423         | 1759         | 1972         | 1973         | HSA/kex2        |
| 318        | 2909         | pSAC35:HSA.HDRMI8 <sub>2</sub>     | Mature HSA fused downstream of the HSA/kex2 leader sequence and upstream of HDRMI82.   | pSAC35            | 1592         | 1424         | 1760         | 1974         | 1975         | HSA/kex2        |
| 319        | 2910         | pSAC35:HSA.RegIV                   | Mature HSA fused downstream of the HSA/kex2 leader sequence and upstream of RegIV.   | pSAC35            | 1593         | 1425         | 1761         | 1976         | 1977         | HSA/kex2        |

**Table 2**

| Fusion No. | Construct ID | Construct Name                    | Description   | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence           |
|------------|--------------|-----------------------------------|---|-------------------|--------------|--------------|--------------|--------------|--------------|---------------------------|
| 320        | 2915         | pC4:HSA#65.humancalcitonin.C1-G33 | Modified HSA leader #65 followed by mature HSA and C98 through G130 of SEQ ID NO: 1762.                                     | pC4               | 1594         | 1426         | 1762         | 1978         | 1979         | Modified HSA #65          |
| 321        | 2930         | pC4.MPIF.Insulin(GYG).HSA         | Insulin is downstream of an MPIF signal peptide and upstream of mature HSA.   | pC4               | 1595         | 1427         | 1763         | 1980         | 1981         | MPIF                      |
| 322        | 2931         | pC4.HSA.Insulin(GYG)              | Synthetic gene coding for a mature single-chain insulin fused downstream of the modified HSA A14 leader and mature HSA.     | pC4               | 1596         | 1428         | 1764         | 1982         | 1983         | Modified HSA (A14) leader |
| 323        | 2942         | pSac35.TA57.Insulin(GYG).HSA      | The TA57 Propeptide fused to a single chain insulin (GYG), and then mature HSA.   | pScNHSA           | 1597         | 1429         | 1765         | 1984         | 1985         | TA57 propeptide           |
| 324        | 2943         | pSAC35:HSA.T7.T7.T7.4-L98         | Dimer construct- HSA/kex2 leader followed by mature HSA followed by two copies of T7 peptide (SEQ ID NO:1766) of Tumstatin. | pScNHSA           | 1598         | 1430         | 1766         | 1986         | 1987         | HSA/kex2                  |
| 325        | 2944         | pSAC:HSA.T8.T8.K69-S95            | HSA/kex2 leader followed by mature HSA followed by two copies of T8 peptide (SEQ ID NO: 1767) of Tumstatin                  | pScNHSA           | 1599         | 1431         | 1767         | 1988         | 1989         | HSA/kex2                  |



**Table 2**

| Fusion No. | Construct ID | Construct Name                          | Description   | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence |
|------------|--------------|---|---|-------------------|-------------|-------------|-------------|-------------|-------------|-----------------|
| 326        | 2945         | pSAC35:GLP-1(7-36(A8S)):GLP-1(7-36).HSA | Amino acids H98 to R127 of preproglucagon (SEQ ID NO:1250) is mutated at position 99 from alanine to serine (hereinafter, this particular mutant is referred to as GLP-1(7-36(A8S)), which is fused downstream from the HSA/kex2 signal sequence and upstream from GLP-1(7-36), and mature HSA.             | pScCHSA           | 1235        | 1220        | 1250        | 1269        | 1270        | HSA/kex2        |
| 327        | 2946         | pSAC:T1249(x2).HSA                      | This dimer represents the wild type sequence for T1249. Both dimers have been yeast codon optimized. The second dimer was optimized to be different from the first (at the wobble position) to ensure good amplification. Construct has the HSA/kex2 leader followed by T1249 dimer followed by mature HSA. | pScCHSA           | 1600        | 1432        | 1768        | 1990        | 1991        | HSA/kex2        |
| 328        | 2947         | pSAC:CKb-188(x2).HSA                    | Invertase signal peptide followed by amino acids G28-N93 of full length CKβ1 (SEQ IDNO:1769), tandemly repeated, followed by mature HSA.  | pSAC35            | 1601        | 1433        | 1769        | 1992        | 1993        | invertase       |
| 329        | 2964         | pSAC35:GLP-1(7-36)x2.HSA                | GLP-1(7-36) is tandemly repeated as a dimer and fused downstream from the HSA/kex2 leader sequence and upstream from mature HSA.  | pSAC35            | 1236        | 1221        | 1251        | 1271        | 1272        | HSA/kex2        |
| 330        | 2965         | pC4:MPIFspP.PTH(1-34).HSA               | MPIF signal peptide followed by 34 amino acids of PTH followed by mature HSA.   | pC4               | 1602        | 1434        | 1770        | 1994        | 1995        | MPIF            |

**Table 2**

| Fusion No. | Construct ID | Construct Name                              | Description  | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence    |
|------------|--------------|---|--|-------------------|-------------|-------------|-------------|-------------|-------------|--------------------|
| 331        | 2966         | pEE12:MPIFsp.PTH(1-34).HSA                  | MPIF signal peptide followed by 34 amino acids of PTH followed by mature HSA.  | PEE12.1           | 1603        | 1435        | 1771        | 1996        | 1997        | MPIF               |
| 332        | 2982         | pSAC35:GLP-1(7-36(A8G)).GLP-1(7-36).HSA     | GLP-1(7-36(A8G)) (SEQ ID NO:1808) is fused downstream from the HSA/kex2 signal sequence and upstream from GLP-1(7-36) and mature HSA.                        | pScCHSA           | 1237        | 1222        | 1252        | 1273        | 1274        | HSA/kex2           |
| 333        | 2983         | pC4.HSA.Growth Hormone.F27-F-217            | Modified (A14) HSA leader followed by mature HSA followed by F27 through F217 of growth hormone (corresponding to amino acids F1 to F191 of SEQ ID NO:1772). | pC4               | 1604        | 1436        | 1772        | 1998        | 1999        | Modified HSA (A14) |
| 334        | 2986         | pSac35.y3SP.TA57PP.I nsulin(GYG).HSA        | The TA57 Propeptide fused to a single chain insulin (GYG), and then mature HSA.  | pScCHSA           | 1605        | 1437        | 1773        | 2000        | 2001        | TA57 propeptide    |
| 335        | 3025         | pSAC35:INU.Insulin.H SA                     | Inulinase signal peptide is fused upstream of single chain insulin (GYG) and HSA.  | pScCHSA           | 1606        | 1438        | 1774        | 2002        | 2003        | inulinase          |
| 336        | 3027         | pSAC35:INV.GLP-1(7-36A8G)x2.HSA             | Invertase signal peptide followed by GLP-1(7-36(A8G)) (SEQ ID NO:1808) tandemly repeated as a dimer, followed by mature HSA.                                 | pSAC35            | 1607        | 1439        | 1775        | 2004        | 2005        | invertase          |
| 337        | 3028         | pSAC35:INV.GLP-1(7-36(A8G)).GLP-1(7-36).HSA | Invertase signal peptide followed by GLP-1(7-36(A8G)) (SEQ ID NO:1808), then GLP-1(7-36(A8G)), and then mature HSA.  | pSAC35            | 1608        | 1440        | 1776        | 2006        | 2007        | invertase          |

**Table 2**

| Fusion No. | Construct ID | Construct Name                                  | Description  | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence               |
|------------|--------------|---|--|-------------------|--------------|--------------|--------------|--------------|--------------|-------------------------------|
| 338        | 3045         | pSAC35:DeltaKex.GLP-1(7-36A8G)x2.HSA            | HSA/kex2 signal sequence, minus the last six amino acids of the leader, is fused to GLP-1(7-36(A8G)) (SEQ ID NO:1808) which is tandemly repeated as a dimer, followed by mature HSA. | pSAC35            | 1609         | 1440         | 1776         | 2008         | 2009         | HSA/kex2 last six amino acids |
| 339        | 3046         | pSAC35:Delta Kex.GLP-1(7-36A8G).GLP-1(7-36).HSA | HSA/kex2 signal sequence, minus the last six amino acids of the leader, is fused to GLP-1(7-36(A8G)) (SEQ ID NO:1808), GLP-1(7-36), and mature HSA.                                  | pSAC35            | 1610         | 1440         | 1776         | 2010         | 2011         | HSA/kex2 last six amino acids |
| 340        | 3047         | pSAC35: HSA.Tum5                                | Full length HSA fused to the Tum5 peptide (SEQ ID NO:1779) of Tumstatin.   | pScNHSA           | 1611         | 1443         | 1779         | 2012         | 2013         | HSA                           |
| 341        | 3048         | pSAC35: Tum5.HSA.                               | The Tum5 peptide (SEQ ID NO:1780) of Tumstatin is fused to HSA and HSA/kex2 leader.  | pScCHSA           | 1612         | 1444         | 1780         | 2014         | 2015         | HSA/kex2                      |
| 342        | 3049         | pC4.HSA.HCE1P80.D92-L229                        | Amino acids D92 to L229 of HCE1P80 are fused downstream of the full length HSA.  | pC4               | 1613         | 1445         | 1781         | 2016         | 2017         | HSA                           |
| 343        | 3050         | pC4.HSA.HCE1P80.A20-L229                        | Amino acids A20-L229 of HCE1P80 are fused downstream of the full length human HSA  | pC4               | 1614         | 1446         | 1782         | 2018         | 2019         | HSA                           |
| 344        | 3051         | pSAC35.HSA.HCE1P80.D92-L229                     | Amino acids D92 to L229 of HCE1P80, a member of the C1q family of proteins, are fused downstream of the full length human HSA  | pSAC35            | 1615         | 1447         | 1783         | 2020         | 2021         | HSA                           |
| 345        | 3052         | pSAC35.HSA.HCE1P80.A20-L229                     | Amino acids A20-L229 of HCE1P80 are fused downstream of the full length human HSA  | pSAC35            | 1616         | 1448         | 1784         | 2022         | 2023         | HSA                           |

**Table 2**

| Fusion No. | Construct ID | Construct Name                    | Description   | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence |
|------------|--------------|-----------------------------------|---|-------------------|-------------|-------------|-------------|-------------|-------------|-----------------|
| 346        | 3053         | pC4.HSA.HDALV07.K101-N244         | The globular domain of adiponectin (amino acids K101-N244) has been inserted downstream of full length human HSA  | pC4               | 1617        | 1449        | 1785        | 2024        | 2025        | HSA             |
| 347        | 3055         | pSAC35.HSA.HDALV07(GD)            | Full length HSA followed by amino acids K101-N244 of HDALV07(GD)/Adiponectin.   | pSAC35            | 1618        | 1450        | 1786        | 2026        | 2027        | HSA             |
| 348        | 3056         | pSAC35.HSA.HDALV07.MP             | Full length HSA followed by amino acids Q18 to N244 of HDALV07.   | pSAC35            | 1619        | 1451        | 1787        | 2028        | 2029        | HSA             |
| 349        | 3066         | pSAC35:CKB-1d8.GLP-1(7-36).HSA    | Invertase signal peptide followed by amino acids G28-N93 of full length CKβ1 (SEQ IDNO:1788), followed by GLP-1(7-36), followed by mature HSA.                            | pScCHSA           | 1620        | 1452        | 1788        | 2030        | 2031        | invertase       |
| 350        | 3069         | pSAC35:INU.GLP-1(7-36(A8G))x2.HSA | The inulinase signal sequence is fused to GLP-1(7-36(A8G)) (SEQ ID NO:1808), which is tandemly repeated as a dimer and fused to mature HSA.                               | pSAC35            | 1621        | 1453        | 1789        | 2032        | 2033        | inulinase       |
| 351        | 3070         | pSAC35:KT.GLP-1(7-36(A8G))x2.HSA  | GLP-1(7-36(A8G)) (SEQ ID NO:1808) is tandemly repeated as a dimer and fused upstream from mature HSA and downstream from the killer toxin signal sequence.                | pSAC35            | 1280        | 1281        | 1282        | 1283        | 1284        | Killer toxin    |
| 352        | 3071         | pSAC35:MAF.GLP-1(7-36(A8G))x2.HSA | The yeast mating factor α-1 (hereinafter MFα-1) signal sequence is fused to tandemly repeated copies of GLP-1(7-36(A8G)) (SEQ ID NO:1808), which are fused to mature HSA. | pSAC35            | 1622        | 1454        | 1790        | 2034        | 2035        | MFα-1           |

**Table 2**

| Fusion No. | Construct ID | Construct Name                              | Description  | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence  |
|------------|--------------|---|--|-------------------|-------------|-------------|-------------|-------------|-------------|------------------|
| 353        | 3072         | pSAC35:AP.GLP-1(7-36(A8G))x2.HSA            | The acid phosphatase signal sequence is fused to tandemly repeated copies of GLP-1(7-36(A8G)) (SEQ ID NO:1808), which are fused to mature HSA.               | pSAC35            | 1623        | 1455        | 1791        | 2036        | 2037        | Acid phosphatase |
| 354        | 3085         | pSAC35:MAF.GLP-1(7-36(A8G)).GLP-1(7-36).HSA | The yeast mating factor $\alpha$ -1 (hereinafter MF $\alpha$ -1) signal sequence is fused to GLP-1(7-36(A8G)) (SEQ ID NO:1808), GLP-1(7-36), and mature HSA. | pSAC35            | 1624        | 1456        | 1792        | 2038        | 2039        | MF $\alpha$ -1   |
| 355        | 3086         | pSAC35:INU.GLP-1(7-36(A8G)).GLP-1(7-36).HSA | The inulinase signal sequence is fused to GLP-1(7-36(A8G)) (SEQ ID NO:1808), GLP-1(7-36), and mature HSA.  | pSAC35            | 1625        | 1457        | 1793        | 2040        | 2041        | inulinase        |
| 356        | 3087         | pSAC35:AP.GLP-1(7-36(A8G)).GLP-1(7-36).HSA  | The acid phosphatase signal sequence is fused to GLP-1(7-36(A8G)) (SEQ ID NO:1808), GLP-1(7-36), and mature HSA.   | pSAC35            | 1626        | 1458        | 1794        | 2042        | 2043        | Acid phosphatase |
| 357        | 3088         | pSAC35:HSA.C-Peptide                        | HSA/kex2 signal peptide, followed by HSA, followed by the C-Peptide sequence.  | pSAC35            | 1627        | 1459        | 1795        | 2044        | 2045        | HSA/kex2         |
| 358        | 3105         | pSAC35:INV.t9HCC-1.G28-N93:spc.HSA          | Invertase signal peptide followed by amino acids G28 to N93 of HCC-1 fused upstream of a spacer and mature HSA.  | pSAC35            | 1628        | 1460        | 1796        | 2046        | 2047        | invertase        |
| 359        | 3106         | pSACHSA.HCBOG68                             | mature HCBOG68 fused downstream of mature HSA and the HSA/kex2 leader sequence.  | pSAC35            | 1629        | 1461        | 1797        |             |             | HSA/kex2         |
| 360        | 3108         | pSAC35HSA.PYY                               | Mature PYY fused downstream of mature HSA and the HSA/kex2 leader.   | pSAC35            | 1630        | 1462        | 1798        |             |             | HSA/kex2         |

**Table 2**

| Fusion No. | Construct ID | Construct Name                        | Description  | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence |
|------------|--------------|---------------------------------------|--|-------------------|-------------|-------------|-------------|-------------|-------------|-----------------|
| 361        | 3109         | pSAC35:HSA.PYY3-36                    | HSA/kex2 leader followed by mature HSA and then PYY3-36 (SEQ ID NO:1799).  | pSAC35            | 1631        | 1463        | 1799        |             |             | HSA/kex2        |
| 362        | 3117         | pC4:PYY3-36/HSA                       | HSA leader followed by PYY3-36 (SEQ ID NO:1800) and mature HSA.  | pC4               | 1632        | 1464        | 1800        | 2048        | 2049        | HSA             |
| 363        | 3118         | pSAC35:PYY3-36/HSA                    | HSA/kex2 leader followed by PYY3-36 (SEQ ID NO:1801) and mature HSA.   | pSAC35            | 1633        | 1465        | 1801        | 2050        | 2051        | HSA/kex2        |
| 364        | 3119         | pSAC35:BNP/HSA                        | HSA/kex2 leader followed by BNP and mature HSA.  | pSAC35            | 1634        | 1466        | 1802        | 2052        | 2053        | HSA/kex2        |
| 365        | 3124         | pSAC35:INV.CKB1.P2 9-N93:HSA          | Invertase signal peptide followed by amino acids 29 to 93 of full length ckbeta1 fused to N-terminus of HSA.   | pSAC35            | 1635        | 1467        | 1803        | 2054        | 2055        | invertase       |
| 366        | 3125         | pSAC35:INV.CKb-1.R27-N93:HSA          | Invertase signal peptide followed by amino acids 27 to 93 of full length ckbeta1 fused to N-terminus of HSA.   | pSAC35            | 1636        | 1468        | 1804        | 2056        | 2057        | invertase       |
| 367        | 3133         | pSAC35.ySP.TA57PP.Insulin(GYG).HSA    | Variant TA57 propeptide leader followed by single chain insulin, followed by mature HSA.   | pSAC35            | 1637        | 1469        | 1805        | 2058        | 2059        | TA57 variant 1  |
| 368        | 3134         | pSAC35.ySP.TA57PP+S. Insulin(GYG).HSA | Variant TA57 propeptide leader followed by single chain insulin, followed by mature HSA.   | pSAC35            | 1638        | 1470        | 1806        | 2060        | 2061        | TA57 variant 2  |
| 369        | 3139         | pSAC35:INV.CKB1.G2 8-N93.DAHK.HSA     | Invertase signal peptide followed by amino acids G28-N93 of full length CKβ1 (see, e.g, SEQ IDNO:1788), followed by a 16 amino acid linker derived from the N-terminus of HSA, followed by mature HSA. | pSAC35            | 1639        | 1471        | 1807        | 2062        | 2063        | invertase       |

**Table 2**

| Fusion No. | Construct ID | Construct Name                    | Description   | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence |
|------------|--------------|-----------------------------------|---|-------------------|-------------|-------------|-------------|-------------|-------------|-----------------|
| 370        | 3140         | pSAC35:GLP1(mut)DA HK.HSA         | GLP-1(7-36(A8G)) (SEQ ID NO:1808) is linked to mature HSA by a 16 amino acid linker derived from the N-terminus of HSA. The HSA/kex2 signal sequence is used. | pSAC35            | 1640        | 1472        | 1808        | 2064        | 2065        | HSA/kex2        |
| 371        | 3141         | pSAC35:Wnt10b/HSA                 | HSA/kex2 leader followed by amino acids N29 to K389 of Wnt10b followed by mature HSA.   | pSAC35            | 1641        | 1473        | 1809        | 2066        | 2067        | HSA/kex2        |
| 372        | 3142         | pSAC35:Wnt11/HSA                  | HSA/kex2 leader followed by mature Wnt11 followed by mature HSA.  | pSAC35            | 1642        | 1474        | 1810        | 2068        | 2069        | HSA/kex2        |
| 373        | 3143         | pSAC35:herstatin/HSA              | HSA/kex2 leader followed by amino acids T23 to G419 of herstatin followed by mature HSA.  | pSAC35            | 1643        | 1475        | 1811        | 2070        | 2071        | HSA/kex2        |
| 374        | 3144         | pSAC35:adrenomedullin (27-52)/HSA | HSA/kex2 leader followed by amino acids 27-52 of adrenomedullin followed by mature HSA.   | pSAC35            | 1644        | 1476        | 1812        | 2072        | 2073        | HSA/kex2        |
| 375        | 3149         | pSAC35:HSA.C-peptide tandem       | Full length HSA fused to amino acids E7 to Q37 of SEQ ID NO:1813, tandemly repeated.  | pSAC35            | 1645        | 1477        | 1813        | 2074        | 2075        | HSA             |
| 376        | 3152         | pSAC35:INV.CKB1.Me t.R27-N93.HSA  | Invertase signal peptide followed by a Met, followed by amino acids R27-N93 of full length CKβ1, followed by mature HSA.                                      | pSAC35            | 1646        | 1478        | 1814        | 2076        | 2077        | invertase       |
| 377        | 3153         | pSAC35:INV.CKB1.Me t.S26-N93.HSA  | Invertase signal peptide followed by a Met, followed by amino acids S26-N93 of full length CKβ1, followed by mature HSA.                                      | pSAC35            | 1647        | 1479        | 1815        | 2078        | 2079        | invertase       |

**Table 2**

| Fusion No. | Construct ID | Construct Name   | Description  | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence |
|------------|--------------|--|--|-------------------|--------------|--------------|--------------|--------------|--------------|-----------------|
| 378        | 3154         | pSAC35:INV.CKB1.Me<br>t.S25-N93.HSA                        | Invertase signal peptide followed by a Met, followed by amino acids S25-N93 of full length CKβ1, followed by mature HSA. | pSAC35            | 1648         | 1480         | 1816         | 2080         | 2081         | invertase       |
| 379        | 3155         | pSAC35:INV.CKB1.Me<br>t.G28-N93.HSA                        | Invertase signal peptide followed by a Met, followed by amino acids G28-N93 of full length CKβ1, followed by mature HSA. | pSAC35            | 1649         | 1481         | 1817         | 2082         | 2083         | invertase       |
| 380        | 3156         | pSAC35:INV.CKB1.Me<br>t.P29-N93.HSA                        | Invertase signal peptide followed by a Met, followed by amino acids P29-N93 of full length CKβ1, followed by mature HSA. | pSAC35            | 1650         | 1482         | 1818         | 2084         | 2085         | invertase       |
| 381        | 3163         | pSAC35:HSA.hGH   | HSA/kex2 leader fused upstream of mature HSA and 191 amino acids of hGH.   | pSAC35            | 1303         | 1304         | 1305         |              |              | HSA/kex2        |
| 382        | 3165         | pSAC35:HSA.IFNα<br>also named CID 3165,<br>pSAC35:HSA.IFNα | HSA fused upstream of IFNα and downstream of the HSA/kex2 leader.  | pSAC35            | 1300         | 1301         | 1302         |              |              | HSA/kex2        |
| 383        | 3166         | pC4:MPIF1.A22-<br>N93.HSA                                  | Amino acids A49 to N120 of MPIF (SEQ ID NO:1821) is fused downstream of MPIF signal peptide and upstream of mature HSA.  | pC4               | 1651         | 1483         | 1819         | 2086         | 2087         | MPIF            |
| 384        | 3167         | pC4:HSA.MPIF1.D45-<br>N120                                 | Full length HSA followed by amino acids D45 through N120 of MPIF.  | pC4               | 1652         | 1484         | 1820         | 2088         | 2089         | HSA             |
| 385        | 3168         | PC4:MPIF-1.HSA   | Amino acids D45 through N120 of MPIF fused downstream of the MPIF signal sequence and upstream of mature HSA.            | pC4               | 1653         | 1485         | 1821         | 2090         | 2091         | MPIF            |



**Table 2**

| Fusion No. | Construct ID | Construct Name                           | Description   | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence      |
|------------|--------------|--|---|-------------------|-------------|-------------|-------------|-------------|-------------|----------------------|
| 386        | 3169         | pSAC35:KT.CKB1.G28-N93.HSA               | Killer toxin signal sequence fused upstream of amino acids G28 through N93 of CKB1 (fragment shown as amino acids G1 to N66 of SEQ ID NO:1822) and mature HSA.            | pSAC35            | 1654        | 1486        | 1822        |             |             | Killer toxin         |
| 387        | 3170         | pSAC35:KT.HA.CKB1.G28-N93.HSA            | Killer toxin signal sequence followed by HA dipeptide and amino acids G28 through N93 of CKB1 (fragment shown as amino acids G1 to N66 of SEQ ID NO:1823) and mature HSA. | pSAC35            | 1655        | 1487        | 1823        |             |             | Killer toxin         |
| 388        | 3171         | pSAC35:sCNTF(M1-G185):HSA                | C-terminal deletion of CNTF (amino acids M1 through G185), fused upstream of mature HSA and codon optimized for expression in yeast. HSA/kex2 signal sequence is used.    | pSAC35            | 1656        | 1488        | 1824        | 2092        | 2093        | HSA/kex2             |
| 389        | 3172         | pSAC35:HSA:sCNTF(M1-G185)                | HSA/kex2 signal sequence followed by mature HSA and M1 through G185 of CNTF.  | pSAC35            | 1657        | 1489        | 1825        | 2094        | 2095        | HSA/kex2             |
| 390        | 3184         | pC4:HSA.NOGOR.C27-C309                   | Full length HSA followed by amino acids C27 to C309 of the NOGO receptor.   | pC4               | 1658        | 1490        | 1826        | 2096        | 2097        | HSA                  |
| 391        | 3185         | pC4.NOGOR.M1-C309.HSA                    | Amino acids M1-C309 of NOGO receptor fused upstream of mature HSA.  | pC4               | 1659        | 1491        | 1827        | 2098        | 2099        | Native NOGO receptor |
| 392        | 3194         | pC4:HSA(A14)-EPO(A28-D192.G140)codon opt | Codon optimized EPO(A28-D192.G140) fused downstream of mature HSA with a modified HSA (A14) signal sequence.  | pC4               | 1660        | 1492        | 1828        | 2100        | 2101        | modified HSA (A14)   |

**Table 2**

| Fusion No. | Construct ID | Construct Name                           | Description   | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence    |
|------------|--------------|--|---|-------------------|-------------|-------------|-------------|-------------|-------------|--------------------|
| 393        | 3195         | pC4:HSA(S14)-EPO(A28-D192.G140)codon opt | Codon optimized EPO(A28-D192.G140) fused downstream of mature HSA and a modified HSA (S14) signal sequence.                                   | pC4               | 1661        | 1493        | 1829        | 2102        | 2103        | modified HSA (S14) |
| 394        | 3196         | pC4:HSA(G14)-EPO(A28-D192.G140)codon opt | Codon optimized EPO(A28-D192.G140) fused downstream of mature HSA with a modified (G14) HSA signal sequence.                                  | pC4               | 1662        | 1494        | 1830        | 2104        | 2105        | modified (G14)     |
| 395        | 3197         | pC4.MPIF.Insulin(EAE).HSA                | A single-chain insulin is downstream of the MPIF signal peptide and upstream of mature human HSA.   | pC4               | 1663        | 1495        | 1831        |             |             | MPIF               |
| 396        | 3198         | pSac35.INV.insulin(EAE).HSA              | Single-chain insulin is downstream of the invertase signal peptide and upstream of mature human HSA   | pSAC35            | 1664        | 1496        | 1832        |             |             | invertase          |
| 397        | 3202         | pSAC35:API.d8CKb1/HSA                    | HSA/kex2 leader followed by amino acids "API" followed by d8CKb1 and mature HSA. The sequence of delta 8 for CKB1 is shown in SEQ ID NO:1833. | pSAC35            | 1665        | 1497        | 1833        | 2106        | 2107        | HSA/kex2           |
| 398        | 3203         | pSAC35:ASL.d8CKb1/HSA                    | HSA/kex2 leader followed by amino acids "ASL" followed by d8CKb1 and mature HSA.  | pSAC35            | 1666        | 1498        | 1834        | 2108        | 2109        | HSA/kex2           |
| 399        | 3204         | pSAC35:SPY.d8CKb1/HSA                    | HSA/kex2 leader followed by amino acids "SPY" followed by d8CKb1 and mature HSA.  | pSAC35            | 1667        | 1499        | 1835        | 2110        | 2111        | HSA/kex2           |
| 400        | 3205         | pSAC35:MSPY.d8CKb1/HSA                   | HSA/kex2 leader followed by amino acids "MSPY" followed by d8CKb1 and mature HSA.   | pSAC35            | 1668        | 1500        | 1836        | 2112        | 2113        | HSA/kex2           |

**Table 2**

| Fusion No. | Construct ID | Construct Name              | Description   | Expression Vector | SEQ ID NO: Y | SEQ ID NO: X | SEQ ID NO: Z | SEQ ID NO: A | SEQ ID NO: B | Leader Sequence        |
|------------|--------------|-----------------------------|---|-------------------|--------------|--------------|--------------|--------------|--------------|------------------------|
| 401        | 3206         | pSAC35:CPYSC.d8CKb1/HSA     | HSA/kex2 leader followed by a five amino acid linker followed by d8CKb1 and mature HSA.   | pSAC35            | 1669         | 1501         | 1837         | 2114         | 2115         | HSA/kex2               |
| 402        | 3207         | pSAC35:GPY.d8CKb1/HSA       | HSA/kex2 leader followed by amino acids "GPY" followed by d8CKb1 and mature HSA.  | pSAC35            | 1670         | 1502         | 1838         | 2116         | 2117         | HSA/kex2               |
| 403        | 3208         | pSAC35:defensin alpha 1/HSA | Amino acids A65-C94 of defensin alpha 1 fused downstream of the HSA/kex2 leader and upstream of mature HSA.   | pSAC35            | 1285         | 1286         | 1287         | 1288         | 1289         | HSA/kex2               |
| 404        | 3209         | pSAC35:defensin alpha 2/HSA | Amino acids C66-C94 of defensin alpha 2 fused downstream of the HSA/kex2 leader and upstream of mature HSA.   | pSAC35            | 1290         | 1291         | 1292         | 1293         | 1294         | HSA/kex2               |
| 405        | 3210         | pSAC35:defensin alpha 3/HSA | Amino acids 65-94 of SEQ ID NO1297, with A65D and F92I mutations, fused downstream of the HSA/kex2 leader and upstream of mature HSA.               | pSAC35            | 1295         | 1296         | 1297         | 1298         | 1299         | HSA/kex2               |
| 406        | 3232         | pSAC35:CART/HSA             | HSA/kex2 leader followed by processed active cocaine-amphetamine regulated transcript (CART) (amino acids V69 through L116) followed by mature HSA. | pSAC35            | 1671         | 1503         | 1839         | 2118         | 2119         | HSA/kex2               |
| 407        | 3238         | pSAC35:phosphatonin. HSA    | Phosphatonin fused upstream of HSA.   | pSAC35            | 1306         | 1307         | 1308         |              |              | Native phosphatonin in |
| 408        | 3270         | pSAC35:adipokine/HSA        | HSA/kex2 leader followed by adipokine followed by mature HSA.   | pSAC35            | 1672         | 1504         | 1840         | 2120         | 2121         | HSA/kex2               |

**Table 2**

| Fusion No. | Construct ID | Construct Name                          | Description   | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence |
|------------|--------------|---|---|-------------------|-------------|-------------|-------------|-------------|-------------|-----------------|
| 409        | 3272         | pSAC35:INV:{D}8CK{b}1(x2)/HSA           | CKbeta-1 tandem repeat (x2) fusion to the N-terminal HSA. Under the invertase signal peptide.           | pSAC35            | 1673        | 1505        | 1841        | 2122        | 2123        | invertase       |
| 410        | 3274         | pSAC35:P1pal-12.HSA                     | P1pal-12 pepducin peptide fused upstream of mature HSA, and downstream of the HSA/kex2 leader sequence. | pSAC35            | 1334        | 1335        | 1336        |             |             | HSA/kex2        |
| 411        | 3275         | pSAC35:P4pal-10.HSA                     | P4pal-10 pepducin peptide fused upstream of mature HSA, and downstream of the HSA/kex2 leader sequence. | pSAC35            | 1337        | 1338        | 1339        |             |             | HSA/kex2        |
| 412        | 3281         | pSAC35:PY3-36(x2)/HSA                   | PYY3-36 tandem repeat (x2) fused upstream of HSA and downstream of the HSA/kex2 signal peptide.         | pSAC35            | 1674        | 1506        | 1842        | 2124        | 2125        | HSA/kex2        |
| 413        | 3282         | pSAC35:HSA/PYY3-36(x2)                  | PYY3-36 tandem repeat (x2) fused downstream of mature HSA and HSA/kex2 leader.                          | pSAC35            | 1675        | 1507        | 1843        | 2126        | 2127        | HSA/kex2        |
| 414        | 3298         | pSAC35:IL21/HSA                         | Amino acids Q30-S162 of IL-21 fused upstream of mature HSA and downstream of HSA/kex2 leader            | pSAC35            | 2167        | 2157        | 2177        | 2188        | 2189        | HSA/Kex2        |
| 415        | 3307         | pSAC35:IL4/HSA                          | Amino acids H25-S153 of IL-4 fused upstream of mature HSA and downstream of HSA/kex2 leader             | pSAC35            | 2168        | 2158        | 2178        | 2190        | 2191        | HSA/Kex2        |
| 416        | 3309         | pSAC:KT.GLP-1(7-36(A8G))x2.MSA.E25-A608 | Killer toxin leader sequence followed by GLP-1(7-36(A8G)) followed by mature mouse serum albumin.       | pSAC35            | 2170        | 2160        | 2180        | 2194        | 2195        | Killer toxin    |
| 417        | 3312         | pSAC35:hOCIL/HSA                        | HSA/kex2 leader followed by amino acids N20 to V149 of hOCIL followed by mature HSA                     | pSAC35            | 2171        | 2161        | 2181        | 2196        | 2197        | HSA/Kex2        |

**Table 2**

| Fusion No. | Construct ID | Construct Name | Description  | Expression Vector | SEQ ID NO:Y | SEQ ID NO:X | SEQ ID NO:Z | SEQ ID NO:A | SEQ ID NO:B | Leader Sequence |
|------------|--------------|----------------|--|-------------------|-------------|-------------|-------------|-------------|-------------|-----------------|
| 418        | 7777         | T20:HSA        | T20 fused downstream of full length HSA                        | pC4               | 1170        | 1171        | 1172        |             |             | HSA             |
| 419        | 8888         | pC4:BNP.HSA    | Human B-type natriuretic peptide fused upstream of mature HSA. | pC4               | 1275        | 1276        | 1277        | 1278        | 1279        | Native BNP      |
| 420        | 9999         | T1249:HSA      | T1249 fused downstream of full length HSA                      | pC4               | 1173        | 1174        | 1175        |             |             | HSA             |

[0066] Table 2 provides a non-exhaustive list of polynucleotides of the invention comprising, or alternatively consisting of, nucleic acid molecules encoding an albumin fusion protein. The first column, "Fusion No." gives a fusion number to each polynucleotide. Column 2, "Construct ID" provides a unique numerical identifier for each polynucleotide of the invention. The Construct IDs may be used to identify polynucleotides which encode albumin fusion proteins comprising, or alternatively consisting of, a Therapeutic protein portion corresponding to a given Therapeutic Protein:X listed in the corresponding row of Table 1 wherein that Construct ID is listed in column 5. The "Construct Name" column (column 3) provides the name of a given albumin fusion construct or polynucleotide.

[0067] The fourth column in Table 2, "Description" provides a general description of a given albumin fusion construct, and the fifth column, "Expression Vector" lists the vector into which a polynucleotide comprising, or alternatively consisting of, a nucleic acid molecule encoding a given albumin fusion protein was cloned. Vectors are known in the art, and are available commercially or described elsewhere. For example, as described in the Examples, an "expression cassette" comprising, or alternatively consisting of, one or more of (1) a polynucleotide encoding a given albumin fusion protein, (2) a leader sequence, (3) a promoter region, and (4) a transcriptional terminator, may be assembled in a convenient cloning vector and subsequently be moved into an alternative vector, such as, for example, an expression vector including, for example, a yeast expression vector or a mammalian expression vector. In one embodiment, for expression in *S. cerevisiae*, an expression cassette comprising, or alternatively consisting of, a nucleic acid molecule encoding an albumin fusion protein is cloned into pSAC35. In another embodiment, for expression in CHO cells, an expression cassette comprising, or alternatively consisting of, a nucleic acid molecule encoding an albumin fusion protein is cloned into pC4. In a further embodiment, a polynucleotide comprising or alternatively consisting of a nucleic acid molecule encoding the Therapeutic protein portion of an albumin fusion protein is cloned into pC4:HSA. In a still further embodiment, for expression in NS0 cells, an expression cassette comprising, or alternatively consisting of, a nucleic acid molecule encoding an albumin fusion protein is cloned into pEE12. Other useful cloning and/or expression vectors will be known to the skilled artisan and are within the scope of the invention.

[0068] Column 6, "SEQ ID NO:Y," provides the full length amino acid sequence of the albumin fusion protein of the invention. In most instances, SEQ ID NO:Y shows the unprocessed form of the albumin fusion protein encoded – in other words, SEQ ID NO:Y

shows the signal sequence, a HSA portion, and a therapeutic portion all encoded by the particular construct. Specifically contemplated by the present invention are all polynucleotides that encode SEQ ID NO:Y. When these polynucleotides are used to express the encoded protein from a cell, the cell's natural secretion and processing steps produces a protein that lacks the signal sequence listed in columns 4 and/or 11 of Table 2. The specific amino acid sequence of the listed signal sequence is shown later in the specification or is well known in the art. Thus, most preferred embodiments of the present invention include the albumin fusion protein produced by a cell (which would lack the leader sequence shown in columns 4 and/or 11 of Table 2). Also most preferred are polypeptides comprising SEQ ID NO:Y without the specific leader sequence listed in columns 4 and/or 11 of Table 2. Compositions comprising these two preferred embodiments, including pharmaceutical compositions, are also preferred. Moreover, it is well within the ability of the skilled artisan to replace the signal sequence listed in columns 4 and/or 11 of Table 2 with a different signal sequence, such as those described later in the specification to facilitate secretion of the processed albumin fusion protein.

**[0069]** The seventh column, "SEQ ID NO:X," provides the parent nucleic acid sequence from which a polynucleotide encoding a Therapeutic protein portion of a given albumin fusion protein may be derived. In one embodiment, the parent nucleic acid sequence from which a polynucleotide encoding a Therapeutic protein portion of an albumin fusion protein may be derived comprises the wild type gene sequence encoding a Therapeutic protein shown in Table 1. In an alternative embodiment, the parent nucleic acid sequence from which a polynucleotide encoding a Therapeutic protein portion of an albumin fusion protein may be derived comprises a variant or derivative of a wild type gene sequence encoding a Therapeutic protein shown in Table 1, such as, for example, a synthetic codon optimized variant of a wild type gene sequence encoding a Therapeutic protein.

**[0070]** The eighth column, "SEQ ID NO:Z," provides a predicted translation of the parent nucleic acid sequence (SEQ ID NO:X). This parent sequence can be a full length parent protein used to derive the particular construct, the mature portion of a parent protein, a variant or fragment of a wildtype protein, or an artificial sequence that can be used to create the described construct. One of skill in the art can use this amino acid sequence shown in SEQ ID NO:Z to determine which amino acid residues of an albumin fusion protein encoded by a given construct are provided by the therapeutic protein. Moreover, it is well within the ability of the skilled artisan to use the sequence shown as SEQ ID NO:Z to derive the construct

described in the same row. For example, if SEQ ID NO:Z corresponds to a full length protein, but only a portion of that protein is used to generate the specific CID, it is within the skill of the art to rely on molecular biology techniques, such as PCR, to amplify the specific fragment and clone it into the appropriate vector.

**[0071]** Amplification primers provided in columns 9 and 10, “SEQ ID NO:A” and “SEQ ID NO:B” respectively, are exemplary primers used to generate a polynucleotide comprising or alternatively consisting of a nucleic acid molecule encoding the Therapeutic protein portion of a given albumin fusion protein. In one embodiment of the invention, oligonucleotide primers having the sequences shown in columns 9 and/or 10 (SEQ ID NOS:A and/or B) are used to PCR amplify a polynucleotide encoding the Therapeutic protein portion of an albumin fusion protein using a nucleic acid molecule comprising or alternatively consisting of the nucleotide sequence provided in column 7 (SEQ ID NO:X) of the corresponding row as the template DNA. PCR methods are well-established in the art. Additional useful primer sequences could readily be envisioned and utilized by those of ordinary skill in the art.

**[0072]** In an alternative embodiment, oligonucleotide primers may be used in overlapping PCR reactions to generate mutations within a template DNA sequence. PCR methods are known in the art.

**[0073]** As shown in Table 3, certain albumin fusion constructs disclosed in this application have been deposited with the ATCC®.



**Table 3**

| Construct ID | Construct Name   | ATCC Deposit No./ Date    |
|--------------|--|---------------------------|
| 1642         | pSAC35:GCSF.T31-P204.HSA   | PTA-3767<br>Oct. 5, 2001  |
| 1643         | pSAC35:HSA.GCSF.T31-P204   | PTA-3766<br>Oct. 5, 2001  |
| 1812         | pSAC35:IL2.A21-T153.HSA  | PTA-3759<br>Oct. 4, 2001  |
| 1941         | pC4:HSA/PTH84(junctioned)  | PTA-3761<br>Oct. 4, 2001  |
| 1949         | pC4:PTH.S1-Q84/HSA (junctioned)  | PTA-3762<br>Oct. 4, 2001  |
| 1966         | pC4:EPO.M1-D192.HSA<br>also named pC4:EPOM1-D192.HSA                     | PTA-3771<br>Oct. 5, 2001  |
| 1981         | pC4.HSA-EPO.A28-D192   | PTA-3770<br>Oct. 5, 2001  |
| 1997         | pEE12.1:EPOM1-D192.HSA   | PTA-3768<br>Oct. 5, 2001  |
| 2030         | pSAC35.ycoIL-2.A21-T153.HSA  | PTA-3757<br>Oct. 4, 2001  |
| 2031         | pSAC35.HSA.ycoIL-2.A21-T153  | PTA-3758<br>Oct. 4, 2001  |
| 2053         | pEE12:IFNb-HSA<br>also named pEE12.1:IFN $\beta$ -HSA                    | PTA-3764<br>Oct. 4, 2001  |
| 2054         | pEE12:HSA-IFNb   | PTA-3941<br>Dec. 19, 2001 |
| 2249         | pSAC35:IFN $\alpha$ 2-HSA<br>also named pSAC23:IFN $\alpha$ 2-HSA        | PTA-3763<br>Oct. 4, 2001  |
| 2250         | pSAC35:HSA.INSULIN(GYG)<br>also named<br>pSAC35.HSA.INSULING(GYG).F1-N62 | PTA-3916<br>Dec. 07, 2001 |
| 2255         | pSAC35:INSULIN(GYG).HSA<br>also named pSAC35.INSULING(GYG).F1-N62.HSA    | PTA-3917<br>Dec. 07, 2001 |
| 2276         | pSAC35:HSA.INSULIN(GGG)<br>also named pSAC35.HSA.INSULING(GGG).F1-N58    | PTA-3918<br>Dec. 07, 2001 |
| 2298         | pEE12.1:EPO.R140G.HSA  | PTA-3760<br>Oct. 4, 2001  |

| Construct ID | Construct Name  | ATCC Deposit No./ Date       |
|--------------|---|------------------------------|
| 2294         | pC4:EPO.R140G.HSA<br>also named pC4.EPO.R1406.HSA   | PTA-3742<br>Sept. 28, 2001   |
| 2325         | pC4.EPO:M1-D192.HSA.Codon opt.  | PTA-3773<br>Oct. 5, 2001     |
| 2343         | pSAC35.INV-IFNA2.HSA  | PTA-3940<br>Dec. 19, 2001    |
| 2363         | pC4.GCSF.HSA.EPO.A28-D192   | PTA-3740<br>Sept. 28, 2001   |
| 2373         | pC4.GCSF.HSA.EPO.A28-D192.R140G   | PTA-3741<br>Sept. 28, 2001   |
| 2381         | pC4:HSA-IFNa2(C17-E181)   | PTA-3942<br>Dec. 19, 2001    |
| 2382         | pC4:IFNa2-HSA   | PTA-3939<br>Dec. 19, 2001    |
| 2387         | pC4:EPO(G140)-HSA-GCSF.T31-P204   | PTA-3919<br>Dec. 11, 2001    |
| 2414         | pC4.EPO:M1-D192copt.HSA.GCSF.T31-P204<br>also named<br>pC4.EPO:M1-D192copt.HAS.GCSF.T31-P204      | PTA-3924<br>Dec. 12, 2001    |
| 2441         | pEE12.EPO:M1-D192copt.HSA.GCSF.T31-P204<br>also named:<br>pEE12.EPO:M1-D192copt.HAS.GCSF.T31-P204 | PTA-3923<br>Dec. 12, 2001    |
| 2492         | pC4.IFNb(deltaM22).HSA  | PTA-3943<br>Dec. 19, 2001    |
| 3070         | pSAC35:KT.GLP-1(7-36(A8G))x2.HSA  | PTA-4671<br>Sept. 16, 2002   |
| 3165         | pSAC35:HSA.IFNa<br>also named CID 3165, pSAC35:HSA.INFa   | PTA-4670<br>Sept. 16, 2002   |
| 3163         | pSAC35:HSA.hGH  | PTA-4770<br>October 22, 2002 |

[0074] It is possible to retrieve a given albumin fusion construct from the deposit by techniques known in the art and described elsewhere herein (see, Example 40). The ATCC is located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA. The ATCC deposits were made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedure.

[0075] In a further embodiment of the invention, an “expression cassette” comprising, or alternatively consisting of one or more of (1) a polynucleotide encoding a given albumin fusion protein, (2) a leader sequence, (3) a promoter region, and (4) a transcriptional terminator can be moved or “subcloned” from one vector into another. Fragments to be subcloned may be generated by methods well known in the art, such as, for example, PCR amplification (e.g., using oligonucleotide primers having the sequence shown in SEQ ID NO:A or B), and/or restriction enzyme digestion.

[0076] In preferred embodiments, the albumin fusion proteins of the invention are capable of a therapeutic activity and/or biologic activity corresponding to the therapeutic activity and/or biologic activity of the Therapeutic protein corresponding to the Therapeutic protein portion of the albumin fusion protein listed in the corresponding row of Table 1. In further preferred embodiments, the therapeutically active protein portions of the albumin fusion proteins of the invention are fragments or variants of the protein encoded by the sequence shown in SEQ ID NO:X column of Table 2, and are capable of the therapeutic activity and/or biologic activity of the corresponding Therapeutic protein.

***Non-human albumin fusion proteins of growth hormone.***

[0077] In one embodiment, the albumin fusion proteins of the invention comprise one or more Serum Albumin proteins of a non-human animal species, fused in tandem and in-frame either at the N-terminus or the C-terminus to one or more Growth Hormone proteins of the same non-human animal species. Non-human Serum Albumin and Growth Hormone proteins are well known in the art and available in public databases. For example, Table 4 presents accession numbers corresponding to non-human Serum Albumin sequences (column 2) and non-human Growth Hormone sequences (column 3) found in GenBank. In a preferred embodiment, a Serum Albumin protein from a non-human animal species listed in Table 4 is fused to a Growth Hormone protein from the same non-human animal species.

[0078] In a specific embodiment, the albumin fusion protein of the invention comprises one or more *Bos taurus* Serum Albumin proteins listed in Table 4, column 2, fused in tandem and in-frame either at the N-terminus or the C-terminus to one or more *Bos taurus* Growth Hormone proteins listed in Table 4, column 3.

[0079] Fusion proteins comprising fragments or variants of non-human Serum Albumin, such as, for example, the mature form of Serum Albumin, are also encompassed by the invention. Fusion proteins comprising fragments or variants of non-human Growth

Hormone proteins, such as, for example, the mature form of Growth Hormone, are also encompassed by the invention. Preferably the non-human Growth Hormone fragments and variants retain growth hormone activity.

**[0080]** Polynucleotides of the invention comprise, or alternatively consist of, one or more nucleic acid molecules encoding a non-human albumin fusion protein described above. For example, the polynucleotides can comprise, or alternatively consist of, one or more nucleic acid molecules that encode a Serum Albumin protein from a non-human animal species listed in Table 4, column 1 (such as, for example, the non-human Serum Albumin reference sequences listed in Table 4, column 2) fused in tandem and in-frame either 5' or 3' to a polynucleotide that comprises, or alternatively consists of, one or more nucleic acid molecules encoding the non-human Growth Hormone protein of the corresponding non-human animal species (for example, the Growth Hormone reference sequences listed in Table 4, column 3).

**[0081]** The above-described non-human albumin fusion proteins are encompassed by the invention, as are host cells and vectors containing these polynucleotides. In one embodiment, a non-human albumin fusion protein encoded by a polynucleotide as described above has extended shelf life. In an additional embodiment, a non-human albumin fusion protein encoded by a polynucleotide described above has a longer serum half-life and/or more stabilized activity in solution (or in a pharmaceutical composition) *in vitro* and/or *in vivo* than the corresponding unfused Growth Hormone molecule.

**[0082]** The present invention also encompasses methods of preventing, treating, or ameliorating a disease or disorder in a non-human animal species. In certain embodiments, the present invention encompasses a method of treating a veterinary disease or disorder comprising administering to a non-human animal species in which such treatment, prevention or amelioration is desired an albumin fusion protein of the invention that comprises a Growth Hormone portion corresponding to a Growth Hormone protein (or fragment or variant thereof) in an amount effective to treat, prevent or ameliorate the disease or disorder. Veterinary diseases and/or disorders which may be treated, prevented, or ameliorated include growth disorders (such as, for example, pituitary dwarfism), shin soreness, obesity, growth hormone-responsive dermatosis, dilated cardiomyopathy, eating disorders, reproductive disorders, and endocrine disorders.

**[0083]** Non-human albumin fusion proteins of the invention may also be used to promote healing of skin wounds, corneal injuries, bone fractures, and injuries of joints,

tendons, or ligaments.

**[0084]** Non-human albumin fusion proteins of the invention may also be used to increase milk production in lactating animals. In a preferred embodiment, the lactating animal is a dairy cow.

**[0085]** Non-human albumin fusion proteins of the invention may also be used to improve body condition in aged animals.

**[0086]** Non-human albumin fusion proteins of the invention may also be used to increase fertility, pregnancy rates, and reproductive success in domesticated animals.

**[0087]** Non-human albumin fusion proteins of the invention may also be used to improve the lean-to-fat ratio in animals raised for consumption, as well as to improve appetite, and increase body size and growth rate.

**Table 4**

| <b>Non-Human Species</b> | <b>Non-Human Serum Albumin Reference Sequence(s): GenBank Protein Accession Nos.</b> | <b>Non-Human Growth Hormone Reference Sequence(s): GenBank Protein Accession Nos.</b>  |
|--------------------------|--|--|
| Bos taurus               | ABBOS, CAA76847, P02769, CAA41735, 229552, AAA51411                                  | STBO, BAA06379, A29864, AAF28806, AAF28805, AAF28804, P01246, AAF03132, AAC63901, AAB92549, A36506, I45901, JC1316, CAA23445, CAA00787, CAA00598, AAA30547, AAA30546, AAA30545, AAA30544, AAA30543, AAA30542     |
| Sus scrofa               | P08835, CAA30970, AAA30988   | STPG, PC1017, AAB29947, AAB84359, I46585, I46584, PC1063, A01516, AAB17619, 226829, 225740, CAA37411, CAA00592, AAA73478, AAA73477, CAA00356, AAA31046, AAA31045, AAA31044, AA30543                              |
| Equus caballus           | ABHOS, AAG40944, P35747, CAA52194  | STHO, P01245, AAD25992, 227704, AAA21027   |
| Ovis aries               | ABSHS, P14639, CAA34903  | STSH, AAB24467, AAC48679, 228487, 223932, CAA34098, CAA31063, CAA00828, AAA31527   |
| Salmo salar              | ABONS2, ABONS2, CAA36643, CAA43187   | STONC, P07064, Q07221, P48096, P10814, P10607, I51186, S03709, JS0179, A23154, S06489, CAA42431, AAB29165, AAB24612, Q91221, Q91222, CAA43942, CAA32481, 738042, 224555, CAA00427, AAA50757, AAA49558, AAA49555, |

| <b>Non-Human Species</b> | <b>Non-Human Serum Albumin Reference Sequence(s): GenBank Protein Accession Nos.</b> | <b>Non-Human Growth Hormone Reference Sequence(s): GenBank Protein Accession Nos.</b>                  |
|--------------------------|--|--|
|                          |  | AAA49553, AAA49401, AAA49406, AAA49403, AAA49402   |
| Gallus gallus            | ABCHS, P19121, CAA43098  | BAB62262, BAB69037, AAK95643, A60509, AAG01029, BAA01365, P08998, 226895, CAA31127, CAA35619, AAA48780 |
| Felis catus              | P49064, S57632, CAA59279, JC4660   | JC4632, P46404, AAC00073, AAA96142, AAA67294   |
| Canis familiaris         | P49822, S29749, CAB64867, CAA76841, AAB30434   | P33711, I46145, AAF89582, AAF21502, AAD43366, S35790, AAB34229, CAA80601                               |

### ***Polypeptide and Polynucleotide Fragments and Variants***

#### ***Fragments***

**[0088]** The present invention is further directed to fragments of the Therapeutic proteins described in Table 1, albumin proteins, and/or albumin fusion proteins of the invention.

**[0089]** The present invention is also directed to polynucleotides encoding fragments of the Therapeutic proteins described in Table 1, albumin proteins, and/or albumin fusion proteins of the invention.

**[0090]** Even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the Therapeutic protein, albumin protein, and/or albumin fusion protein of the invention, other Therapeutic activities and/or functional activities (e.g., biological activities, ability to multimerize, ability to bind a ligand) may still be retained. For example, the ability of polypeptides with N-terminal deletions to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptides generally will be retained when less than the majority of the residues of the complete polypeptide are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a mutein with a large number of deleted N-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides

composed of as few as six amino acid residues may often evoke an immune response.

**[0091]** Accordingly, fragments of a Therapeutic protein corresponding to a Therapeutic protein portion of an albumin fusion protein of the invention, include the full length protein as well as polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the reference polypeptide (i.e., a Therapeutic protein referred to in Table 1, or a Therapeutic protein portion of an albumin fusion protein encoded by a polynucleotide or albumin fusion construct described in Table 2). In particular, N-terminal deletions may be described by the general formula m to q, where q is a whole integer representing the total number of amino acid residues in a reference polypeptide (e.g., a Therapeutic protein referred to in Table 1, or a Therapeutic protein portion of an albumin fusion protein of the invention, or a Therapeutic protein portion of an albumin fusion protein encoded by a polynucleotide or albumin fusion construct described in Table 2), and m is defined as any integer ranging from 2 to q minus 6. Polynucleotides encoding these polypeptides are also encompassed by the invention.

**[0092]** In addition, fragments of serum albumin polypeptides corresponding to an albumin protein portion of an albumin fusion protein of the invention, include the full length protein as well as polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the reference polypeptide (i.e., serum albumin, or a serum albumin portion of an albumin fusion protein encoded by a polynucleotide or albumin fusion construct described in Table 2). In preferred embodiments, N-terminal deletions may be described by the general formula m to 585, where 585 is a whole integer representing the total number of amino acid residues in mature human serum albumin (SEQ ID NO:1038), and m is defined as any integer ranging from 2 to 579. Polynucleotides encoding these polypeptides are also encompassed by the invention. In additional embodiments, N-terminal deletions may be described by the general formula m to 609, where 609 is a whole integer representing the total number of amino acid residues in full length human serum albumin (SEQ ID NO:1094), and m is defined as any integer ranging from 2 to 603. Polynucleotides encoding these polypeptides are also encompassed by the invention.

**[0093]** Moreover, fragments of albumin fusion proteins of the invention, include the full length albumin fusion protein as well as polypeptides having one or more residues deleted from the amino terminus of the albumin fusion protein (e.g., an albumin fusion protein encoded by a polynucleotide or albumin fusion construct described in Table 2; or an albumin fusion protein having the amino acid sequence disclosed in column 6 of Table 2). In

particular, N-terminal deletions may be described by the general formula m to q, where q is a whole integer representing the total number of amino acid residues in the albumin fusion protein, and m is defined as any integer ranging from 2 to q minus 6. Polynucleotides encoding these polypeptides are also encompassed by the invention.

**[0094]** Also as mentioned above, even if deletion of one or more amino acids from the N-terminus or C-terminus of a reference polypeptide (e.g., a Therapeutic protein; serum albumin protein; or albumin fusion protein of the invention) results in modification or loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind a ligand) and/or Therapeutic activities may still be retained. For example the ability of polypeptides with C-terminal deletions to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking the N-terminal and/or C-terminal residues of a reference polypeptide retains Therapeutic activity can readily be determined by routine methods described herein and/or otherwise known in the art.

**[0095]** The present invention further provides polypeptides having one or more residues deleted from the carboxy terminus of the amino acid sequence of a Therapeutic protein corresponding to a Therapeutic protein portion of an albumin fusion protein of the invention (e.g., a Therapeutic protein referred to in Table 1, or a Therapeutic protein portion of an albumin fusion protein encoded by a polynucleotide or albumin fusion construct described in Table 2). In particular, C-terminal deletions may be described by the general formula 1 to n, where n is any whole integer ranging from 6 to q minus 1, and where q is a whole integer representing the total number of amino acid residues in a reference polypeptide (e.g., a Therapeutic protein referred to in Table 1, or a Therapeutic protein portion of an albumin fusion protein encoded by a polynucleotide or albumin fusion construct described in Table 2). Polynucleotides encoding these polypeptides are also encompassed by the invention.

**[0096]** In addition, the present invention provides polypeptides having one or more residues deleted from the carboxy terminus of the amino acid sequence of an albumin protein corresponding to an albumin protein portion of an albumin fusion protein of the invention (e.g., serum albumin or an albumin protein portion of an albumin fusion protein encoded by a polynucleotide or albumin fusion construct described in Table 2). In particular, C-terminal



deletions may be described by the general formula 1 to n, where n is any whole integer ranging from 6 to 584, where 584 is the whole integer representing the total number of amino acid residues in mature human serum albumin (SEQ ID NO:1038) minus 1. Polynucleotides encoding these polypeptides are also encompassed by the invention. In particular, C-terminal deletions may be described by the general formula 1 to n, where n is any whole integer ranging from 6 to 608, where 608 is the whole integer representing the total number of amino acid residues in serum albumin (SEQ ID NO:1094) minus 1. Polynucleotides encoding these polypeptides are also encompassed by the invention.

**[0097]** Moreover, the present invention provides polypeptides having one or more residues deleted from the carboxy terminus of an albumin fusion protein of the invention. In particular, C-terminal deletions may be described by the general formula 1 to n, where n is any whole integer ranging from 6 to q minus 1, and where q is a whole integer representing the total number of amino acid residues in an albumin fusion protein of the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

**[0098]** In addition, any of the above described N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted reference polypeptide. The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m to n of a reference polypeptide (e.g., a Therapeutic protein referred to in Table 1, or a Therapeutic protein portion of an albumin fusion protein of the invention, or a Therapeutic protein portion encoded by a polynucleotide or albumin fusion construct described in Table 2, or serum albumin (e.g., SEQ ID NO:1038), or an albumin protein portion of an albumin fusion protein of the invention, or an albumin protein portion encoded by a polynucleotide or albumin fusion construct described in Table 2, or an albumin fusion protein, or an albumin fusion protein encoded by a polynucleotide or albumin fusion construct of the invention) where n and m are integers as described above. Polynucleotides encoding these polypeptides are also encompassed by the invention.

**[0099]** The present application is also directed to proteins containing polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a reference polypeptide sequence (e.g., a Therapeutic protein referred to in Table 1, or a Therapeutic protein portion of an albumin fusion protein of the invention, or a Therapeutic protein portion encoded by a polynucleotide or albumin fusion construct described in Table 2, or serum albumin (e.g., SEQ ID NO: 1038), or an albumin protein portion of an albumin fusion protein of the invention, or

an albumin protein portion encoded by a polynucleotide or albumin fusion construct described in Table 2, or an albumin fusion protein, or an albumin fusion protein encoded by a polynucleotide or albumin fusion construct of the invention) set forth herein, or fragments thereof. In preferred embodiments, the application is directed to proteins comprising polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to reference polypeptides having the amino acid sequence of N- and C-terminal deletions as described above. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0100] Preferred polypeptide fragments of the invention are fragments comprising, or alternatively, consisting of, an amino acid sequence that displays a Therapeutic activity and/or functional activity (e.g. biological activity) of the polypeptide sequence of the Therapeutic protein or serum albumin protein of which the amino acid sequence is a fragment.

[0101] Other preferred polypeptide fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

### ***Variants***

[0102] "Variant" refers to a polynucleotide or nucleic acid differing from a reference nucleic acid or polypeptide, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the reference nucleic acid or polypeptide.

[0103] As used herein, "variant", refers to a Therapeutic protein portion of an albumin fusion protein of the invention, albumin portion of an albumin fusion protein of the invention, or albumin fusion protein of the invention differing in sequence from a Therapeutic protein (e.g. see "therapeutic" column of Table 1), albumin protein, and/or albumin fusion protein, respectively, but retaining at least one functional and/or therapeutic property thereof as described elsewhere herein or otherwise known in the art. Generally, variants are overall very similar, and, in many regions, identical to the amino acid sequence of the Therapeutic protein corresponding to a Therapeutic protein portion of an albumin fusion protein, albumin protein corresponding to an albumin protein portion of an albumin fusion protein, and/or albumin fusion protein. Nucleic acids encoding these variants are also encompassed by the invention.

[0104] The present invention is also directed to proteins which comprise, or alternatively consist of, an amino acid sequence which is at least 80%, 85%, 90%, 95%, 96%,

97%, 98%, 99% or 100%, identical to, for example, the amino acid sequence of a Therapeutic protein corresponding to a Therapeutic protein portion of an albumin fusion protein of the invention (e.g., the amino acid sequence of a Therapeutic protein:X disclosed in Table 1; or the amino acid sequence of a Therapeutic protein portion of an albumin fusion protein encoded by a polynucleotide or albumin fusion construct described in Table 1 and 2, or fragments or variants thereof), albumin proteins corresponding to an albumin protein portion of an albumin fusion protein of the invention (e.g., the amino acid sequence of an albumin protein portion of an albumin fusion protein encoded by a polynucleotide or albumin fusion construct described in Table 1 and 2; the amino acid sequence shown in SEQ ID NO: 1038; or fragments or variants thereof), and/or albumin fusion proteins. Fragments of these polypeptides are also provided (e.g., those fragments described herein). Further polypeptides encompassed by the invention are polypeptides encoded by polynucleotides which hybridize to the complement of a nucleic acid molecule encoding an albumin fusion protein of the invention under stringent hybridization conditions (e.g., hybridization to filter bound DNA in 6X Sodium chloride/Sodium citrate (SSC) at about 45 degrees Celsius, followed by one or more washes in 0.2X SSC, 0.1% SDS at about 50 - 65 degrees Celsius), under highly stringent conditions (e.g., hybridization to filter bound DNA in 6X sodium chloride/Sodium citrate (SSC) at about 45 degrees Celsius, followed by one or more washes in 0.1X SSC, 0.2% SDS at about 68 degrees Celsius), or under other stringent hybridization conditions which are known to those of skill in the art (see, for example, Ausubel, F.M. et al., eds., 1989 *Current protocol in Molecular Biology*, Green publishing associates, Inc., and John Wiley & Sons Inc., New York, at pages 6.3.1 - 6.3.6 and 2.10.3). Polynucleotides encoding these polypeptides are also encompassed by the invention.

**[0105]** By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, or substituted with another amino acid. These alterations of the reference sequence may occur at the amino- or carboxy-terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more

contiguous groups within the reference sequence.

**[0106]** As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence of an albumin fusion protein of the invention or a fragment thereof (such as a Therapeutic protein portion of the albumin fusion protein or an albumin portion of the albumin fusion protein), can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci.6:237-245 (1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is expressed as percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

**[0107]** If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C- terminal residues of the subject sequence.

**[0108]** For example, a 90 amino acid residue subject sequence is aligned with a 100

residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

**[0109]** The variant will usually have at least 75 % (preferably at least about 80%, 90%, 95% or 99%) sequence identity with a length of normal HA or Therapeutic protein which is the same length as the variant. Homology or identity at the nucleotide or amino acid sequence level is determined by BLAST (Basic Local Alignment Search Tool) analysis using the algorithm employed by the programs blastp, blastn, blastx, tblastn and tblastx (Karlin *et al.*, Proc. Natl. Acad. Sci. USA 87: 2264-2268 (1990) and Altschul, J. Mol. Evol. 36: 290-300 (1993), fully incorporated by reference) which are tailored for sequence similarity searching.

**[0110]** The approach used by the BLAST program is to first consider similar segments between a query sequence and a database sequence, then to evaluate the statistical significance of all matches that are identified and finally to summarize only those matches which satisfy a preselected threshold of significance. For a discussion of basic issues in similarity searching of sequence databases, see Altschul *et al.*, (Nature Genetics 6: 119-129 (1994)) which is fully incorporated by reference. The search parameters for histogram, descriptions, alignments, expect (i.e., the statistical significance threshold for reporting matches against database sequences), cutoff, matrix and filter are at the default settings. The default scoring matrix used by blastp, blastx, tblastn, and tblastx is the BLOSUM62 matrix (Henikoff *et al.*, Proc. Natl. Acad. Sci. USA 89: 10915-10919 (1992), fully incorporated by reference). For blastn, the scoring matrix is set by the ratios of M (i.e., the reward score for a

pair of matching residues) to N (i.e., the penalty score for mismatching residues), wherein the default values for M and N are 5 and -4, respectively. Four blastn parameters may be adjusted as follows: Q=10 (gap creation penalty); R=10 (gap extension penalty); wink=1 (generates word hits at every wink<sup>th</sup> position along the query); and gapw=16 (sets the window width within which gapped alignments are generated). The equivalent Blastp parameter settings were Q=9; R=2; wink=1; and gapw=32. A Bestfit comparison between sequences, available in the GCG package version 10.0, uses DNA parameters GAP=50 (gap creation penalty) and LEN=3 (gap extension penalty) and the equivalent settings in protein comparisons are GAP=8 and LEN=2.

**[0111]** The polynucleotide variants of the invention may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, polypeptide variants in which less than 50, less than 40, less than 30, less than 20, less than 10, or 5-50, 5-25, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host, such as, yeast or *E. coli*).

**[0112]** In a preferred embodiment, a polynucleotide of the invention which encodes the albumin portion of an albumin fusion protein is optimized for expression in yeast or mammalian cells. In a further preferred embodiment, a polynucleotide of the invention which encodes the Therapeutic protein portion of an albumin fusion protein is optimized for expression in yeast or mammalian cells. In a still further preferred embodiment, a polynucleotide encoding an albumin fusion protein of the invention is optimized for expression in yeast or mammalian cells.

**[0113]** In an alternative embodiment, a codon optimized polynucleotide which encodes a Therapeutic protein portion of an albumin fusion protein does not hybridize to the wild type polynucleotide encoding the Therapeutic protein under stringent hybridization conditions as described herein. In a further embodiment, a codon optimized polynucleotide which encodes an albumin portion of an albumin fusion protein does not hybridize to the wild type polynucleotide encoding the albumin protein under stringent hybridization conditions as described herein. In another embodiment, a codon optimized polynucleotide which encodes

an albumin fusion protein does not hybridize to the wild type polynucleotide encoding the Therapeutic protein portion or the albumin protein portion under stringent hybridization conditions as described herein.

**[0114]** In an additional embodiment, a polynucleotide which encodes a Therapeutic protein portion of an albumin fusion protein does not comprise, or alternatively consist of, the naturally occurring sequence of that Therapeutic protein. In a further embodiment, a polynucleotide which encodes an albumin protein portion of an albumin fusion protein does not comprise, or alternatively consist of, the naturally occurring sequence of albumin protein. In an alternative embodiment, a polynucleotide which encodes an albumin fusion protein does not comprise, or alternatively consist of, the naturally occurring sequence of a Therapeutic protein portion or the albumin protein portion.

**[0115]** Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985)). These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

**[0116]** Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the polypeptide of the present invention without substantial loss of biological function. As an example, Ron et al. (J. Biol. Chem. 268: 2984-2988 (1993)) reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988).)

**[0117]** Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem. 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either

[binding or biological activity]." In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

**[0118]** Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

**[0119]** Thus, the invention further includes polypeptide variants which have a functional activity (e.g., biological activity and/or therapeutic activity). In one embodiment, the invention provides variants of albumin fusion proteins that have a functional activity (e.g., biological activity and/or therapeutic activity) that corresponds to one or more biological and/or therapeutic activities of the Therapeutic protein corresponding to the Therapeutic protein portion of the albumin fusion protein. In another embodiment, the invention provides variants of albumin fusion proteins that have a functional activity (e.g., biological activity and/or therapeutic activity) that corresponds to one or more biological and/or therapeutic activities of the Therapeutic protein corresponding to the Therapeutic protein portion of the albumin fusion protein. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as to have little effect on activity. Polynucleotides encoding such variants are also encompassed by the invention.

**[0120]** In preferred embodiments, the variants of the invention have conservative substitutions. By "conservative substitutions" is intended swaps within groups such as replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

**[0121]** Guidance concerning how to make phenotypically silent amino acid substitutions is provided, for example, in Bowie et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," Science 247:1306-1310 (1990), wherein



the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

**[0122]** The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

**[0123]** The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. See Cunningham and Wells, *Science* 244:1081-1085 (1989). The resulting mutant molecules can then be tested for biological activity.

**[0124]** As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly. Besides conservative amino acid substitution, variants of the present invention include (i) polypeptides containing substitutions of one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) polypeptides containing substitutions of one or more of the amino acid residues having a substituent group, or (iii) polypeptides which have been fused with or chemically conjugated to another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), (iv) polypeptide containing additional amino acids, such as, for

example, an IgG Fc fusion region peptide . Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

[0125] For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. See Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).

[0126] In specific embodiments, the polypeptides of the invention comprise, or alternatively, consist of, fragments or variants of the amino acid sequence of an albumin fusion protein, the amino acid sequence of a Therapeutic protein and/or human serum albumin, wherein the fragments or variants have 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, amino acid residue additions, substitutions, and/or deletions when compared to the reference amino acid sequence. In preferred embodiments, the amino acid substitutions are conservative. Nucleic acids encoding these polypeptides are also encompassed by the invention.

[0127] The polypeptide of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as post-translational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative,

covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, *PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES*, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); *POST-TRANSLATIONAL COVALENT MODIFICATION OF PROTEINS*, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., *Meth. Enzymol.* 182:626-646 (1990); Rattan et al., *Ann. N.Y. Acad. Sci.* 663:48-62 (1992)).

### ***Functional activity***

**[0128]** "A polypeptide having functional activity" refers to a polypeptide capable of displaying one or more known functional activities associated with the full-length, pro-protein, and/or mature form of a Therapeutic protein. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a polypeptide for binding) to an anti-polypeptide antibody], immunogenicity (ability to generate antibody which binds to a specific polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide.

**[0129]** "A polypeptide having biological activity" refers to a polypeptide exhibiting activity similar to, but not necessarily identical to, an activity of a Therapeutic protein of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the polypeptide of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention).

**[0130]** In preferred embodiments, an albumin fusion protein of the invention has at least one biological and/or therapeutic activity associated with the Therapeutic protein portion (or fragment or variant thereof) when it is not fused to albumin.

**[0131]** The albumin fusion proteins of the invention can be assayed for functional activity (e.g., biological activity) using or routinely modifying assays known in the art, as well as assays described herein. Additionally, one of skill in the art may routinely assay fragments of a Therapeutic protein corresponding to a Therapeutic protein portion of an albumin fusion protein, for activity using assays referenced in its corresponding row of Table 1 (e.g., in column 3 of Table 1). Further, one of skill in the art may routinely assay fragments of an albumin protein corresponding to an albumin protein portion of an albumin fusion protein, for activity using assays known in the art and/or as described in the Examples section below.

**[0132]** For example, in one embodiment where one is assaying for the ability of an albumin fusion protein to bind or compete with a Therapeutic protein for binding to an anti-Therapeutic polypeptide antibody and/or anti-albumin antibody, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

**[0133]** In a preferred embodiment, where a binding partner (e.g., a receptor or a ligand) of a Therapeutic protein is identified, binding to that binding partner by an albumin fusion protein which comprises that Therapeutic protein as the Therapeutic protein portion of the fusion can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky et al., *Microbiol. Rev.* 59:94-123 (1995). In another embodiment, the ability of physiological correlates of an albumin fusion protein to bind to a substrate(s) of the Therapeutic polypeptide corresponding to the Therapeutic protein portion of the fusion can be routinely assayed using techniques known in the art.

**[0134]** In an alternative embodiment, where the ability of an albumin fusion protein to

multimerize is being evaluated, association with other components of the multimer can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky et al., *supra*.

**[0135]** In preferred embodiments, an albumin fusion protein comprising all or a portion of an antibody that binds a Therapeutic protein, has at least one biological and/or therapeutic activity (e.g., to specifically bind a polypeptide or epitope) associated with the antibody that binds a Therapeutic protein (or fragment or variant thereof) when it is not fused to albumin. In other preferred embodiments, the biological activity and/or therapeutic activity of an albumin fusion protein comprising all or a portion of an antibody that binds a Therapeutic protein is the inhibition (i.e., antagonism) or activation (i.e., agonism) of one or more of the biological activities and/or therapeutic activities associated with the polypeptide that is specifically bound by antibody that binds a Therapeutic protein.

**[0136]** Albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be characterized in a variety of ways. In particular, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be assayed for the ability to specifically bind to the same antigens specifically bound by the antibody that binds a Therapeutic protein corresponding to the Therapeutic protein portion of the albumin fusion protein using techniques described herein or routinely modifying techniques known in the art.

**[0137]** Assays for the ability of the albumin fusion proteins (e.g., comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) to (specifically) bind a specific protein or epitope may be performed in solution (e.g., Houghten, *Bio/Techniques* 13:412-421(1992)), on beads (e.g., Lam, *Nature* 354:82-84 (1991)), on chips (e.g., Fodor, *Nature* 364:555-556 (1993)), on bacteria (e.g., U.S. Patent No. 5,223,409), on spores (e.g., Patent Nos. 5,571,698; 5,403,484; and 5,223,409), on plasmids (e.g., Cull et al., *Proc. Natl. Acad. Sci. USA* 89:1865-1869 (1992)) or on phage (e.g., Scott and Smith, *Science* 249:386-390 (1990); Devlin, *Science* 249:404-406 (1990); Cwirla et al., *Proc. Natl. Acad. Sci. USA* 87:6378-6382 (1990); and Felici, *J. Mol. Biol.* 222:301-310 (1991)) (each of these references is incorporated herein in its entirety by reference). Albumin fusion proteins comprising at least a fragment or variant of a Therapeutic antibody may also be assayed for their specificity and affinity for a specific protein or epitope using or routinely modifying techniques described herein or otherwise known in the art.

**[0138]** The albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be assayed for cross-reactivity with other antigens (e.g., molecules that have sequence/structure conservation with the molecule(s) specifically bound by the antibody that binds a Therapeutic protein (or fragment or variant thereof) corresponding to the Therapeutic protein portion of the albumin fusion protein of the invention) by any method known in the art.

**[0139]** Immunoassays which can be used to analyze (immunospecific) binding and cross-reactivity include, but are not limited to, competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), “sandwich” immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, and protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

**[0140]** Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (*e.g.*, EDTA, PMSF, aprotinin, sodium vanadate), adding the albumin fusion protein of the invention (*e.g.*, comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) to the cell lysate, incubating for a period of time (*e.g.*, 1 to 4 hours) at 40 degrees C, adding sepharose beads coupled to an anti-albumin antibody, for example, to the cell lysate, incubating for about an hour or more at 40 degrees C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the albumin fusion protein to immunoprecipitate a particular antigen can be assessed by, *e.g.*, western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the albumin fusion protein to an antigen and decrease the background (*e.g.*, pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

[0141] Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (*e.g.*, 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (*e.g.*, PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (*e.g.*, PBS-Tween 20), applying the albumin fusion protein of the invention (diluted in blocking buffer) to the membrane, washing the membrane in washing buffer, applying a secondary antibody (which recognizes the albumin fusion protein, *e.g.*, an anti-human serum albumin antibody) conjugated to an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase) or radioactive molecule (*e.g.*,  $^{32}\text{P}$  or  $^{125}\text{I}$ ) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

[0142] ELISAs comprise preparing antigen, coating the well of a 96-well microtiter plate with the antigen, washing away antigen that did not bind the wells, adding the albumin fusion protein (*e.g.*, comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) of the invention conjugated to a detectable compound such as an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase) to the wells and incubating for a period of time, washing away unbound or non-specifically bound albumin fusion proteins, and detecting the presence of the albumin fusion proteins specifically bound to the antigen coating the well. In ELISAs the albumin fusion protein does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes albumin fusion protein) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the albumin fusion protein may be coated to the well. In this case, the detectable molecule could be the antigen conjugated to a detectable compound such as an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase). One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

[0143] The binding affinity of an albumin fusion protein to a protein, antigen, or

epitope and the off-rate of an albumin fusion protein-protein/antigen/epitope interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (*e.g.*,  $^3\text{H}$  or  $^{125}\text{I}$ ) with the albumin fusion protein of the invention in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the albumin fusion protein for a specific protein, antigen, or epitope and the binding off-rates can be determined from the data by Scatchard plot analysis. Competition with a second protein that binds the same protein, antigen or epitope as the albumin fusion protein, can also be determined using radioimmunoassays. In this case, the protein, antigen or epitope is incubated with an albumin fusion protein conjugated to a labeled compound (*e.g.*,  $^3\text{H}$  or  $^{125}\text{I}$ ) in the presence of increasing amounts of an unlabeled second protein that binds the same protein, antigen, or epitope as the albumin fusion protein of the invention.

**[0144]** In a preferred embodiment, BIAcore kinetic analysis is used to determine the binding on and off rates of albumin fusion proteins of the invention to a protein, antigen or epitope. BIAcore kinetic analysis comprises analyzing the binding and dissociation of albumin fusion proteins, or specific polypeptides, antigens or epitopes from chips with immobilized specific polypeptides, antigens or epitopes or albumin fusion proteins, respectively, on their surface.

**[0145]** Antibodies that bind a Therapeutic protein corresponding to the Therapeutic protein portion of an albumin fusion protein may also be described or specified in terms of their binding affinity for a given protein or antigen, preferably the antigen which they specifically bind. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-2} \text{ M}$ ,  $10^{-2} \text{ M}$ ,  $5 \times 10^{-3} \text{ M}$ ,  $10^{-3} \text{ M}$ ,  $5 \times 10^{-4} \text{ M}$ ,  $10^{-4} \text{ M}$ . More preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-5} \text{ M}$ ,  $10^{-5} \text{ M}$ ,  $5 \times 10^{-6} \text{ M}$ ,  $10^{-6} \text{ M}$ ,  $5 \times 10^{-7} \text{ M}$ ,  $10^{-7} \text{ M}$ ,  $5 \times 10^{-8} \text{ M}$  or  $10^{-8} \text{ M}$ . Even more preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-9} \text{ M}$ ,  $10^{-9} \text{ M}$ ,  $5 \times 10^{-10} \text{ M}$ ,  $10^{-10} \text{ M}$ ,  $5 \times 10^{-11} \text{ M}$ ,  $10^{-11} \text{ M}$ ,  $5 \times 10^{-12} \text{ M}$ ,  $10^{-12} \text{ M}$ ,  $5 \times 10^{-13} \text{ M}$ ,  $10^{-13} \text{ M}$ ,  $5 \times 10^{-14} \text{ M}$ ,  $10^{-14} \text{ M}$ ,  $5 \times 10^{-15} \text{ M}$ , or  $10^{-15} \text{ M}$ . In preferred embodiments, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, has an affinity for a given protein or epitope similar to that of the corresponding antibody (not fused to albumin) that binds a Therapeutic protein, taking into account the valency of the albumin fusion protein (comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) and the valency of the corresponding antibody. In addition, assays



described herein (see Examples and Table 1) and otherwise known in the art may routinely be applied to measure the ability of albumin fusion proteins and fragments, variants and derivatives thereof to elicit biological activity and/or Therapeutic activity (either *in vitro* or *in vivo*) related to either the Therapeutic protein portion and/or albumin portion of the albumin fusion protein. Other methods will be known to the skilled artisan and are within the scope of the invention.

### **Albumin**

[0146] As described above, an albumin fusion protein of the invention comprises at least a fragment or variant of a Therapeutic protein and at least a fragment or variant of human serum albumin, which are associated with one another, preferably by genetic fusion.

[0147] An additional embodiment comprises at least a fragment or variant of a Therapeutic protein and at least a fragment or variant of human serum albumin, which are linked to one another by chemical conjugation.

[0148] The terms, human serum albumin (HSA) and human albumin (HA) are used interchangeably herein. The terms, "albumin and "serum albumin" are broader, and encompass human serum albumin (and fragments and variants thereof) as well as albumin from other species (and fragments and variants thereof).

[0149] As used herein, "albumin" refers collectively to albumin protein or amino acid sequence, or an albumin fragment or variant, having one or more functional activities (e.g., biological activities) of albumin. In particular, "albumin" refers to human albumin or fragments thereof (see for example, EP 201 239, EP 322 094 WO 97/24445, WO95/23857) especially the mature form of human albumin as shown in Figure 1 and SEQ ID NO: 1038, or albumin from other vertebrates or fragments thereof, or analogs or variants of these molecules or fragments thereof.

[0150] In preferred embodiments, the human serum albumin protein used in the albumin fusion proteins of the invention contains one or both of the following sets of point mutations with reference to SEQ ID NO: 1038: Leu-407 to Ala, Leu-408 to Val, Val-409 to Ala, and Arg-410 to Ala; or Arg-410 to A, Lys-413 to Gln, and Lys-414 to Gln (see, e.g., International Publication No. WO95/23857, hereby incorporated in its entirety by reference herein). In even more preferred embodiments, albumin fusion proteins of the invention that contain one or both of above-described sets of point mutations have improved

stability/resistance to yeast Yap3p proteolytic cleavage, allowing increased production of recombinant albumin fusion proteins expressed in yeast host cells.

**[0151]** As used herein, a portion of albumin sufficient to prolong the therapeutic activity or shelf-life of the Therapeutic protein refers to a portion of albumin sufficient in length or structure to stabilize or prolong the therapeutic activity of the protein so that the shelf life of the Therapeutic protein portion of the albumin fusion protein is prolonged or extended compared to the shelf-life in the non-fusion state. The albumin portion of the albumin fusion proteins may comprise the full length of the HA sequence as described above, or may include one or more fragments thereof that are capable of stabilizing or prolonging the therapeutic activity. Such fragments may be of 10 or more amino acids in length or may include about 15, 20, 25, 30, 50, or more contiguous amino acids from the HA sequence or may include part or all of specific domains of HA. For instance, one or more fragments of HA spanning the first two immunoglobulin-like domains may be used. In a preferred embodiment, the HA fragment is the mature form of HA.

**[0152]** The albumin portion of the albumin fusion proteins of the invention may be a variant of normal HA. The Therapeutic protein portion of the albumin fusion proteins of the invention may also be variants of the Therapeutic proteins as described herein. The term “variants” includes insertions, deletions and substitutions, either conservative or non conservative, where such changes do not substantially alter one or more of the oncotic, useful ligand-binding and non-immunogenic properties of albumin, or the active site, or active domain which confers the therapeutic activities of the Therapeutic proteins.

**[0153]** In particular, the albumin fusion proteins of the invention may include naturally occurring polymorphic variants of human albumin and fragments of human albumin, for example those fragments disclosed in EP 322 094 (namely HA (P<sub>n</sub>), where n is 369 to 419). The albumin may be derived from any vertebrate, especially any mammal, for example human, cow, sheep, or pig. Non-mammalian albumins include, but are not limited to, hen and salmon. The albumin portion of the albumin fusion protein may be from a different animal than the Therapeutic protein portion.

**[0154]** Generally speaking, an HA fragment or variant will be at least 100 amino acids long, preferably at least 150 amino acids long. The HA variant may consist of or alternatively comprise at least one whole domain of HA, for example domains 1 (amino acids 1-194 of SEQ ID NO: 1038), domain 2 (amino acids 195-387 of SEQ ID NO: 1038), domain 3 (amino acids 388-585 of SEQ ID NO: 1038), domains 1 and 2 (1-387 of SEQ ID NO:

1038), domains 2 and 3 (195-585 of SEQ ID NO: 1038) or domains 1 and 3 (amino acids 1-194 of SEQ ID NO: 1038 and amino acids 388-585 of SEQ ID NO: 1038). Each domain is itself made up of two homologous subdomains namely 1-105, 120-194, 195-291, 316-387, 388-491 and 512-585, with flexible inter-subdomain linker regions comprising residues Lys106 to Glu119, Glu292 to Val315 and Glu492 to Ala511.

[0155] Preferably, the albumin portion of an albumin fusion protein of the invention comprises at least one subdomain or domain of HA or conservative modifications thereof. If the fusion is based on subdomains, some or all of the adjacent linker is preferably used to link to the Therapeutic protein moiety.

#### ***Antibodies that Specifically bind Therapeutic proteins are also Therapeutic proteins***

[0156] The present invention also encompasses albumin fusion proteins that comprise at least a fragment or variant of an antibody that specifically binds a Therapeutic protein disclosed in Table 1. It is specifically contemplated that the term "Therapeutic protein" encompasses antibodies that bind a Therapeutic protein (e.g., as Described in column I of Table 1) and fragments and variants thereof. Thus an albumin fusion protein of the invention may contain at least a fragment or variant of a Therapeutic protein, and/or at least a fragment or variant of an antibody that binds a Therapeutic protein.

#### ***Antibody structure and background***

[0157] The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. *See generally, Fundamental Immunology* Chapters 3-5 (Paul, W., ed., 4th ed. Raven Press, N.Y. (1998)) (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair form the antibody binding site.

[0158] Thus, an intact IgG antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are the same.

[0159] The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. The CDR regions, in general, are the portions of the antibody which make contact with the antigen and determine its specificity. The CDRs from the heavy and the light chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains variable regions comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The variable regions are connected to the heavy or light chain constant region. The assignment of amino acids to each domain is in accordance with the definitions of Kabat *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk *J Mol. Biol.* 196:901-917 (1987); Chothia et al. *Nature* 342:878-883 (1989).

[0160] As used herein, "antibody" refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that specifically binds an antigen (e.g., a molecule containing one or more CDR regions of an antibody). Antibodies that may correspond to a Therapeutic protein portion of an albumin fusion protein include, but are not limited to, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies (e.g., single chain Fvs), Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies specific to antibodies of the invention), and epitope-binding fragments of any of the above (e.g., VH domains, VL domains, or one or more CDR regions).

#### ***Antibodies that bind Therapeutic Proteins***

[0161] The present invention encompasses albumin fusion proteins that comprise at least a fragment or variant of an antibody that binds a Therapeutic Protein (e.g., as disclosed in Table 1) or fragment or variant thereof.

[0162] Antibodies that bind a Therapeutic protein (or fragment or variant thereof) may be from any animal origin, including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, sheep, rabbit, goat, guinea pig, camel, horse, or chicken antibodies. Most preferably, the antibodies are human antibodies. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries and xenomice or other organisms that have been genetically engineered to produce human

antibodies.

**[0163]** The antibody molecules that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. In preferred embodiments, the antibody molecules that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein are IgG1. In other preferred embodiments, the immunoglobulin molecules that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein are IgG2. In other preferred embodiments, the immunoglobulin molecules that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein are IgG4.

**[0164]** Most preferably the antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')<sub>2</sub>, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains.

**[0165]** The antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a Therapeutic protein or may be specific for both a Therapeutic protein as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., *J. Immunol.* 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., *J. Immunol.* 148:1547-1553 (1992).

**[0166]** Antibodies that bind a Therapeutic protein (or fragment or variant thereof) may be bispecific or bifunctional which means that the antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, e.g., Songsivilai & Lachmann *Clin. Exp. Immunol.* 79: 315-321 (1990), Kostelny et al. *J Immunol.* 148:1547 1553 (1992). In addition,

bispecific antibodies may be formed as "diabodies" (Holliger et al. "Diabodies': small bivalent and bispecific antibody fragments" *PNAS USA* 90:6444-6448 (1993)) or "Janusins" (Traunecker et al. "Bispecific single chain molecules (Janusins) target cytotoxic lymphocytes on HIV infected cells" *EMBO J* 10:3655-3659 (1991) and Traunecker et al. "Janusin: new molecular design for bispecific reagents" *Int J Cancer Suppl* 7:51-52 (1992)).

**[0167]** The present invention also provides albumin fusion proteins that comprise, fragments or variants (including derivatives) of an antibody described herein or known elsewhere in the art. Standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding a molecule of the invention, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis which result in amino acid substitutions. Preferably, the variants (including derivatives) encode less than 50 amino acid substitutions, less than 40 amino acid substitutions, less than 30 amino acid substitutions, less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the reference VH domain, VHCDR1, VHCDR2, VHCDR3, VL domain, VLCDR1, VLCDR2, or VLCDR3. In specific embodiments, the variants encode substitutions of VHCDR3. In a preferred embodiment, the variants have conservative amino acid substitutions at one or more predicted non-essential amino acid residues.

**[0168]** Antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein may be described or specified in terms of the epitope(s) or portion(s) of a Therapeutic protein which they recognize or specifically bind. Antibodies which specifically bind a Therapeutic protein or a specific epitope of a Therapeutic protein may also be excluded. Therefore, the present invention encompasses antibodies that specifically bind Therapeutic proteins, and allows for the exclusion of the same. In preferred embodiments, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, binds the same epitopes as the unfused fragment or variant of that antibody itself.

**[0169]** Antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a Therapeutic protein are included. Antibodies that bind polypeptides with at

least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% sequence identity (as calculated using methods known in the art and described herein) to a Therapeutic protein are also included in the present invention. In specific embodiments, antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% sequence identity (as calculated using methods known in the art and described herein) to a Therapeutic protein are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. In preferred embodiments, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, has similar or substantially identical cross reactivity characteristics compared to the fragment or variant of that particular antibody itself.

[0170] Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide encoding a Therapeutic protein under stringent hybridization conditions (as described herein). Antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M. More preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-5}$  M,  $10^{-5}$  M,  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M or  $10^{-8}$  M. Even more preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, or  $10^{-15}$  M. In preferred embodiments, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, has an affinity for a given protein or epitope similar to that of the corresponding antibody (not fused to albumin) that binds a Therapeutic protein, taking into account the valency of the albumin fusion protein

(comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) and the valency of the corresponding antibody.

**[0171]** The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of a Therapeutic protein as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%. In preferred embodiments, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, competitively inhibits binding of a second antibody to an epitope of a Therapeutic protein. In other preferred embodiments, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, competitively inhibits binding of a second antibody to an epitope of a Therapeutic protein by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

**[0172]** Antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention may act as agonists or antagonists of the Therapeutic protein. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described *supra*). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody. In preferred embodiments, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, has similar or substantially similar characteristics with regard to preventing ligand binding and/or preventing receptor activation compared to an un-fused fragment or variant of the antibody that binds the Therapeutic protein.



[0173] The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the Therapeutic proteins (e.g. as disclosed in Table 1). The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res. 58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties). In preferred embodiments, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, have similar or substantially identical agonist or antagonist properties as an un-fused fragment or variant of the antibody that binds the Therapeutic protein.

[0174] Antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention may be used, for example, to purify, detect, and target Therapeutic proteins, including both in *in vitro* and *in vivo* diagnostic and therapeutic methods. For example, the antibodies have utility in immunoassays for qualitatively and quantitatively measuring levels of the Therapeutic protein in biological samples. See, e.g., Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); incorporated by reference herein in its entirety. Likewise, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, may be used, for example, to purify, detect, and

target Therapeutic proteins, including both *in vitro* and *in vivo* diagnostic and therapeutic methods.

[0175] Antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids. Albumin fusion proteins of the invention may also be modified as described above.

#### ***Methods of Producing Antibodies that bind Therapeutic Proteins***

[0176] The antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of-interest can be produced by various procedures well known in the art. For example, a Therapeutic protein may be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen.

Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

[0177] Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681

(Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term “monoclonal antibody” as used herein is not limited to antibodies produced through hybridoma technology. The term “monoclonal antibody” refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

**[0178]** Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. In a non-limiting example, mice can be immunized with a Therapeutic protein or fragment or variant thereof, an albumin fusion protein, or a cell expressing such a Therapeutic protein or fragment or variant thereof or albumin fusion protein. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

**[0179]** Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

**[0180]** Another well known method for producing both polyclonal and monoclonal human B cell lines is transformation using Epstein Barr Virus (EBV). Protocols for generating EBV-transformed B cell lines are commonly known in the art, such as, for example, the protocol outlined in Chapter 7.22 of Current Protocols in Immunology, Coligan et al., Eds., 1994, John Wiley & Sons, NY, which is hereby incorporated in its entirety by reference. The source of B cells for transformation is commonly human peripheral blood, but B cells for transformation may also be derived from other sources including, but not limited to, lymph nodes, tonsil, spleen, tumor tissue, and infected tissues. Tissues are generally made into single cell suspensions prior to EBV transformation. Additionally, steps may be taken to either physically remove or inactivate T cells (e.g., by treatment with cyclosporin A) in B

cell-containing samples, because T cells from individuals seropositive for anti-EBV antibodies can suppress B cell immortalization by EBV.

**[0181]** In general, the sample containing human B cells is inoculated with EBV, and cultured for 3-4 weeks. A typical source of EBV is the culture supernatant of the B95-8 cell line (ATCC #VR-1492). Physical signs of EBV transformation can generally be seen towards the end of the 3-4 week culture period. By phase-contrast microscopy, transformed cells may appear large, clear, hairy and tend to aggregate in tight clusters of cells. Initially, EBV lines are generally polyclonal. However, over prolonged periods of cell cultures, EBV lines may become monoclonal or polyclonal as a result of the selective outgrowth of particular B cell clones. Alternatively, polyclonal EBV transformed lines may be subcloned (e.g., by limiting dilution culture) or fused with a suitable fusion partner and plated at limiting dilution to obtain monoclonal B cell lines. Suitable fusion partners for EBV transformed cell lines include mouse myeloma cell lines (e.g., SP2/0, X63-Ag8.653), heteromyeloma cell lines (human x mouse; e.g., SPAM-8, SBC-H20, and CB-F7), and human cell lines (e.g., GM 1500, SKO-007, RPMI 8226, and KR-4). Thus, the present invention also provides a method of generating polyclonal or monoclonal human antibodies against polypeptides of the invention or fragments thereof, comprising EBV-transformation of human B cells.

**[0182]** Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')<sub>2</sub> fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). F(ab')<sub>2</sub> fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

**[0183]** For example, antibodies that bind to a Therapeutic protein can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the

phage gene III or gene VIII protein. Examples of phage display methods that can be used to make antibodies that bind to a Therapeutic protein include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

**[0184]** As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')<sub>2</sub> fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., BioTechniques 12(6):864-869 (1992); and Sawai et al., AJRI 34:26-34 (1995); and Better et al., Science 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

**[0185]** Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., Methods in Enzymology 203:46-88 (1991); Shu et al., PNAS 90:7995-7999 (1993); and Skerra et al., Science 240:1038-1040 (1988). For some uses, including *in vivo* use of antibodies in humans and *in vitro* detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies et al., (1989) J. Immunol. Methods 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-

human species and a framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., *Nature* 332:323 (1988), which are incorporated herein by reference in their entirety.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, *Molecular Immunology* 28(4/5):489-498 (1991); Studnicka et al., *Protein Engineering* 7(6):805-814 (1994); Roguska. et al., *PNAS* 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

**[0186]** Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

**[0187]** Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which

express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, *Int. Rev. Immunol.* 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; 5,939,598; 6,075,181; and 6,114,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

**[0188]** Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., *Bio/technology* 12:899-903 (1988)).

### ***Polynucleotides Encoding Antibodies***

**[0189]** The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or alternatively, under lower stringency hybridization conditions, e.g., as defined *supra*, to polynucleotides that encode an antibody, preferably, that specifically binds to a Therapeutic protein, and more preferably, an antibody that binds to a polypeptide having the amino acid sequence of a "Therapeutic protein:X" as disclosed in the "SEQ ID NO:Z" column of Table 2.

**[0190]** The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide

sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., *BioTechniques* 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

**[0191]** Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art (See Example 107).

**[0192]** Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

**[0193]** In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well known in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or



more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described *supra*. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed *supra*, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

**[0194]** In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described *supra*, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

**[0195]** Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423-42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* may also be used (Skerra et al., Science 242:1038-1041 (1988)).

### ***Recombinant Expression of Antibodies***

**[0196]** Recombinant expression of an antibody, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody or a single chain antibody), requires construction of an expression vector containing a polynucleotide that encodes the antibody.

Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

**[0197]** The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

**[0198]** A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention *in situ*. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing

antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., *Gene* 45:101 (1986); Cockett et al., *Bio/Technology* 8:2 (1990)).

**[0199]** In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., *EMBO J.* 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, *Nucleic Acids Res.* 13:3101-3109 (1985); Van Heeke & Schuster, *J. Biol. Chem.* 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

**[0200]** In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

**[0201]** In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

**[0202]** In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

**[0203]** For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators,

polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

**[0204]** A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgprt- or apt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, 1993, TIB TECH 11(5):155-215 (1993)); and hygromycin, which confers resistance to hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

**[0205]** The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing

antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

**[0206]** Vectors which use glutamine synthase (GS) or DHFR as the selectable markers can be amplified in the presence of the drugs methionine sulfoximine or methotrexate, respectively. An advantage of glutamine synthase based vectors are the availability of cell lines (e.g., the murine myeloma cell line, NS0) which are glutamine synthase negative. Glutamine synthase expression systems can also function in glutamine synthase expressing cells (e.g. Chinese Hamster Ovary (CHO) cells) by providing additional inhibitor to prevent the functioning of the endogenous gene. A glutamine synthase expression system and components thereof are detailed in PCT publications: WO87/04462; WO86/05807; WO89/01036; WO89/10404; and WO91/06657 which are incorporated in their entireties by reference herein. Additionally, glutamine synthase expression vectors that may be used according to the present invention are commercially available from suppliers, including, for example Lonza Biologics, Inc. (Portsmouth, NH). Expression and production of monoclonal antibodies using a GS expression system in murine myeloma cells is described in Bebbington *et al.*, *Bio/technology* 10:169(1992) and in Biblia and Robinson *Biotechnol. Prog.* 11:1 (1995) which are incorporated in their entireties by reference herein.

**[0207]** The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

**[0208]** Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other

standard technique for the purification of proteins. In addition, the antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

### ***Modifications of Antibodies***

**[0209]** Antibodies that bind a Therapeutic protein or fragments or variants can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the hemagglutinin tag (also called the "HA tag"), which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the "flag" tag.

**[0210]** The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example

of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{111}\text{In}$  or  $^{99}\text{Tc}$ . Other examples of detectable substances have been described elsewhere herein.

**[0211]** Further, an antibody of the invention may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example,  $^{213}\text{Bi}$ . A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

**[0212]** The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF-alpha, TNF-beta, AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al.*, *Int. Immunol.*, 6:1567-1574 (1994)), VEGI (See, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"),



granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

[0213] Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

[0214] Techniques for conjugating such therapeutic moiety to antibodies are well known. See, for example, Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* 62:119-58 (1982).

[0215] Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

[0216] An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

### ***Antibody-albumin fusion***

[0217] Antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention include, but are not limited to, antibodies that bind a Therapeutic protein disclosed in the "Therapeutic Protein X" column of Table 1, or a fragment or variant thereof.

[0218] In specific embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, the VH domain.

In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, one, two or three VH CDRs. In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, the VH CDR1. In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, the VH CDR2. In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, the VH CDR3.

**[0219]** In specific embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, the VL domain. In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, one, two or three VL CDRs. In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, the VL CDR1. In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, the VL CDR2. In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, the VL CDR3.

**[0220]** In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, one, two, three, four, five, or six VH and/or VL CDRs.

**[0221]** In preferred embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein

portion of an albumin fusion protein comprises, or alternatively consists of, an scFv comprising the VH domain of the Therapeutic antibody, linked to the VL domain of the therapeutic antibody by a peptide linker such as (Gly<sub>4</sub>Ser)<sub>3</sub> (SEQ ID NO:1092).

### ***Immunophenotyping***

[0222] The antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein (or fragment or variant thereof) may be utilized for immunophenotyping of cell lines and biological samples. Therapeutic proteins of the present invention may be useful as cell-specific markers, or more specifically as cellular markers that are differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies (or albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies (or albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison *et al.*, *Cell*, 96:737-49 (1999)).

[0223] These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

### ***Characterizing Antibodies that bind a Therapeutic Protein and Albumin Fusion Proteins Comprising a Fragment or Variant of an Antibody that binds a Therapeutic Protein***

[0224] The antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein (or fragment or variant thereof) may be characterized in a variety of ways. In particular, Albumin

fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be assayed for the ability to specifically bind to the same antigens specifically bound by the antibody that binds a Therapeutic protein corresponding to the antibody that binds a Therapeutic protein portion of the albumin fusion protein using techniques described herein or routinely modifying techniques known in the art.

**[0225]** Assays for the ability of the antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein (or fragment or variant thereof) to (specifically) bind a specific protein or epitope may be performed in solution (*e.g.*, Houghten, *Bio/Techniques* 13:412-421(1992)), on beads (*e.g.*, Lam, *Nature* 354:82-84 (1991)), on chips (*e.g.*, Fodor, *Nature* 364:555-556 (1993)), on bacteria (*e.g.*, U.S. Patent No. 5,223,409), on spores (*e.g.*, Patent Nos. 5,571,698; 5,403,484; and 5,223,409), on plasmids (*e.g.*, Cull et al., *Proc. Natl. Acad. Sci. USA* 89:1865-1869 (1992)) or on phage (*e.g.*, Scott and Smith, *Science* 249:386-390 (1990); Devlin, *Science* 249:404-406 (1990); Cwirla et al., *Proc. Natl. Acad. Sci. USA* 87:6378-6382 (1990); and Felici, *J. Mol. Biol.* 222:301-310 (1991)) (each of these references is incorporated herein in its entirety by reference). The antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein (or fragment or variant thereof) may also be assayed for their specificity and affinity for a specific protein or epitope using or routinely modifying techniques described herein or otherwise known in the art.

**[0226]** The albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be assayed for cross-reactivity with other antigens (*e.g.*, molecules that have sequence/structure conservation with the molecule(s) specifically bound by the antibody that binds a Therapeutic protein (or fragment or variant thereof) corresponding to the Therapeutic protein portion of the albumin fusion protein of the invention) by any method known in the art.

**[0227]** Immunoassays which can be used to analyze (immunospecific) binding and cross-reactivity include, but are not limited to, competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, and protein A immunoassays, to name but a few. Such assays are routine and well known in the

art (see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

**[0228]** Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (*e.g.*, EDTA, PMSF, aprotinin, sodium vanadate), adding an antibody of the invention or albumin fusion protein of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein (or fragment or variant thereof) to the cell lysate, incubating for a period of time (*e.g.*, 1 to 4 hours) at 40 degrees C, adding protein A and/or protein G sepharose beads (or beads coated with an appropriate anti-idiotypic antibody or anti-albumin antibody in the case when an albumin fusion protein comprising at least a fragment or variant of a Therapeutic antibody) to the cell lysate, incubating for about an hour or more at 40 degrees C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody or albumin fusion protein of the invention to immunoprecipitate a particular antigen can be assessed by, *e.g.*, western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody or albumin fusion protein to an antigen and decrease the background (*e.g.*, pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

**[0229]** Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (*e.g.*, 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (*e.g.*, PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (*e.g.*, PBS-Tween 20), applying the antibody or albumin fusion protein of the invention (diluted in blocking buffer) to the membrane, washing the membrane in washing buffer, applying a secondary antibody (which recognizes the albumin fusion protein, *e.g.*, an anti-human serum albumin antibody) conjugated to an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase) or radioactive molecule (*e.g.*, <sup>32</sup>P or

<sup>125</sup>I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

[0230] ELISAs comprise preparing antigen, coating the well of a 96-well microtiter plate with the antigen, washing away antigen that did not bind the wells, adding the antibody or albumin fusion protein (comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) of the invention conjugated to a detectable compound such as an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase) to the wells and incubating for a period of time, washing away unbound or non-specifically bound albumin fusion proteins, and detecting the presence of the antibody or albumin fusion proteins specifically bound to the antigen coating the well. In ELISAs the antibody or albumin fusion protein does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody or albumin fusion protein, respectively) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, antibody or the albumin fusion protein may be coated to the well. In this case, the detectable molecule could be the antigen conjugated to a detectable compound such as an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase). One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

[0231] The binding affinity of an albumin fusion protein to a protein, antigen, or epitope and the off-rate of an antibody- or albumin fusion protein-protein/antigen/epitope interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (*e.g.*, <sup>3</sup>H or <sup>125</sup>I) with the antibody or albumin fusion protein of the invention in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody or albumin fusion protein of the invention for a specific protein, antigen, or epitope and the binding off-rates can be determined from the data by Scatchard plot analysis. Competition with a second protein that binds the same protein, antigen or epitope as the antibody or albumin fusion protein, can also be determined using

radioimmunoassays. In this case, the protein, antigen or epitope is incubated with an antibody or albumin fusion protein of the invention conjugated to a labeled compound (*e.g.*,  $^3\text{H}$  or  $^{125}\text{I}$ ) in the presence of increasing amounts of an unlabeled second protein that binds the same protein, antigen, or epitope as the albumin fusion protein of the invention.

[0232] In a preferred embodiment, BIAcore kinetic analysis is used to determine the binding on and off rates of antibody or albumin fusion proteins of the invention to a protein, antigen or epitope. BIAcore kinetic analysis comprises analyzing the binding and dissociation of antibodies, albumin fusion proteins, or specific polypeptides, antigens or epitopes from chips with immobilized specific polypeptides, antigens or epitopes, antibodies or albumin fusion proteins, respectively, on their surface.

### ***Therapeutic Uses***

[0233] The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein), nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein), albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, and nucleic acids encoding such albumin fusion proteins. The antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a Therapeutic protein, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a Therapeutic protein includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

**[0234]** In a specific and preferred embodiment, the present invention is directed to antibody-based therapies which involve administering antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein to an animal, preferably a mammal, and most preferably a human, patient for treating one or more diseases, disorders, or conditions, including but not limited to: neural disorders, immune system disorders, muscular disorders, reproductive disorders, gastrointestinal disorders, pulmonary disorders, cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions., and/or as described elsewhere herein. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (e.g., antibodies directed to the full length protein expressed on the cell surface of a mammalian cell; antibodies directed to an epitope of a Therapeutic protein and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a Therapeutic protein, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a Therapeutic protein includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

**[0235]** A summary of the ways in which the antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be used therapeutically includes binding Therapeutic proteins locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein for diagnostic, monitoring or therapeutic purposes without undue experimentation.



[0236] The antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

[0237] The antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

[0238] It is preferred to use high affinity and/or potent *in vivo* inhibiting and/or neutralizing antibodies against Therapeutic proteins, fragments or regions thereof, (or the albumin fusion protein correlate of such an antibody) for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention, including fragments thereof. Preferred binding affinities include dissociation constants or  $K_d$ 's less than  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M. More preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-5}$  M,  $10^{-5}$  M,  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M or  $10^{-8}$  M. Even more preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, or  $10^{-15}$  M.

### ***Gene Therapy***

[0239] In a specific embodiment, nucleic acids comprising sequences encoding antibodies that bind therapeutic proteins or albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of

a Therapeutic protein, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

[0240] Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described in more detail elsewhere in this application.

#### ***Demonstration of Therapeutic or Prophylactic Activity***

[0241] The compounds or pharmaceutical compositions of the invention are preferably tested *in vitro*, and then *in vivo* for the desired therapeutic or prophylactic activity, prior to use in humans. For example, *in vitro* assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, *in vitro* assays which can be used to determine whether administration of a specific compound is indicated, include *in vitro* cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

#### ***Therapeutic/Prophylactic Administration and Composition***

[0242] The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical composition of the invention. In a preferred embodiment, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

[0243] Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional

appropriate formulations and routes of administration can be selected from among those described herein below.

[0244] Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0245] In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

[0246] In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

[0247] In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*;

Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); see also Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J.Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, e.g., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)).

[0248] Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

[0249] In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox- like peptide which is known to enter the nucleus (see e.g., Joliot et al., Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

[0250] The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered

intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

**[0251]** In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

**[0252]** The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0253] The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a Therapeutic protein can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

[0254] For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

### ***Diagnosis and Imaging***

[0255] Labeled antibodies and derivatives and analogs thereof that bind a Therapeutic protein (or fragment or variant thereof) (including albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein), can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of Therapeutic protein. The invention provides for the detection of aberrant expression of a Therapeutic protein, comprising (a) assaying the expression of the Therapeutic protein in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed Therapeutic protein expression level compared to the standard expression level is indicative of aberrant expression.

[0256] The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the Therapeutic protein in cells or body fluid of an

individual using one or more antibodies specific to the Therapeutic protein or albumin fusion proteins comprising at least a fragment of variant of an antibody specific to a Therapeutic protein, and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed Therapeutic protein gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

**[0257]** Antibodies of the invention or albumin fusion proteins comprising at least a fragment of variant of an antibody specific to a Therapeutic protein can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen et al., J. Cell . Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (<sup>125</sup>I, <sup>121</sup>I), carbon (<sup>14</sup>C), sulfur (<sup>35</sup>S), tritium (<sup>3</sup>H), indium (<sup>112</sup>In), and technetium (<sup>99</sup>Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

**[0258]** One facet of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a Therapeutic protein in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the Therapeutic protein is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the therapeutic protein.

Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

[0259] It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of <sup>99m</sup>Tc. The labeled antibody, antibody fragment, or albumin fusion protein comprising at least a fragment or variant of an antibody that binds a Therapeutic protein will then preferentially accumulate at the location of cells which contain the specific Therapeutic protein. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

[0260] Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

[0261] In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

[0262] Presence of the labeled molecule can be detected in the patient using methods known in the art for *in vivo* scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

[0263] In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument... In another embodiment, the molecule is labeled with a positron emitting metal and is



detected in the patent using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI). Antibodies that specifically detect the albumin fusion protein but not albumin or the therapeutic protein alone are a preferred embodiment. These can be used to detect the albumin fusion protein as described throughout the specification.

### ***Kits***

**[0264]** The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

**[0265]** In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

**[0266]** In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of

the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

[0267] In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

[0268] In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

[0269] The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

[0270] Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

### **Albumin Fusion Proteins**

[0271] The present invention relates generally to albumin fusion proteins and

methods of treating, preventing, or ameliorating diseases or disorders. As used herein, "albumin fusion protein" refers to a protein formed by the fusion of at least one molecule of albumin (or a fragment or variant thereof) to at least one molecule of a Therapeutic protein (or fragment or variant thereof). An albumin fusion protein of the invention comprises at least a fragment or variant of a Therapeutic protein and at least a fragment or variant of human serum albumin, which are associated with one another, preferably by genetic fusion (i.e., the albumin fusion protein is generated by translation of a nucleic acid in which a polynucleotide encoding all or a portion of a Therapeutic protein is joined in-frame with a polynucleotide encoding all or a portion of albumin) or to one another. The Therapeutic protein and albumin protein, once part of the albumin fusion protein, may each be referred to as a "portion", "region" or "moiety" of the albumin fusion protein.

**[0272]** In a preferred embodiment, the invention provides an albumin fusion protein encoded by a polynucleotide or albumin fusion construct described in Table 1 or Table 2. Polynucleotides encoding these albumin fusion proteins are also encompassed by the invention.

**[0273]** Preferred albumin fusion proteins of the invention, include, but are not limited to, albumin fusion proteins encoded by a nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide encoding at least one molecule of albumin (or a fragment or variant thereof) joined in frame to at least one polynucleotide encoding at least one molecule of a Therapeutic protein (or fragment or variant thereof); a nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide encoding at least one molecule of albumin (or a fragment or variant thereof) joined in frame to at least one polynucleotide encoding at least one molecule of a Therapeutic protein (or fragment or variant thereof) generated as described in Table 1, Table 2 or in the Examples; or a nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide encoding at least one molecule of albumin (or a fragment or variant thereof) joined in frame to at least one polynucleotide encoding at least one molecule of a Therapeutic protein (or fragment or variant thereof), further comprising, for example, one or more of the following elements: (1) a functional self-replicating vector (including but not limited to, a shuttle vector, an expression vector, an integration vector, and/or a replication system), (2) a region for initiation of transcription (e.g., a promoter region, such as for example, a regulatable or inducible promoter, a constitutive promoter), (3) a region for termination of transcription, (4) a leader sequence, and (5) a selectable marker.

**[0274]** In one embodiment, the invention provides an albumin fusion protein

comprising, or alternatively consisting of, a Therapeutic protein (e.g., as described in Table 1) and a serum albumin protein. In other embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a biologically active and/or therapeutically active fragment of a Therapeutic protein and a serum albumin protein. In other embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a biologically active and/or therapeutically active variant of a Therapeutic protein and a serum albumin protein. In preferred embodiments, the serum albumin protein component of the albumin fusion protein is the mature portion of serum albumin.

**[0275]** In further embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a Therapeutic protein, and a biologically active and/or therapeutically active fragment of serum albumin. In further embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a Therapeutic protein and a biologically active and/or therapeutically active variant of serum albumin. In preferred embodiments, the Therapeutic protein portion of the albumin fusion protein is the mature portion of the Therapeutic protein.

**[0276]** In further embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a biologically active and/or therapeutically active fragment or variant of a Therapeutic protein and a biologically active and/or therapeutically active fragment or variant of serum albumin. In preferred embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, the mature portion of a Therapeutic protein and the mature portion of serum albumin.

**[0277]** Preferably, the albumin fusion protein comprises HA as the N-terminal portion, and a Therapeutic protein as the C-terminal portion. Alternatively, an albumin fusion protein comprising HA as the C-terminal portion, and a Therapeutic protein as the N-terminal portion may also be used.

**[0278]** In other embodiments, the albumin fusion protein has a Therapeutic protein fused to both the N-terminus and the C-terminus of albumin. In a preferred embodiment, the Therapeutic proteins fused at the N- and C- termini are the same Therapeutic proteins. In an alternative preferred embodiment, the Therapeutic proteins fused at the N- and C- termini are different Therapeutic proteins. In another preferred embodiment, the Therapeutic proteins fused at the N- and C- termini are different Therapeutic proteins which may be used to treat or prevent the same or a related disease, disorder, or condition (e.g. as listed in the "Preferred Indication Y" column of Table 1). In another preferred embodiment, the Therapeutic proteins

fused at the N- and C- termini are different Therapeutic proteins which may be used to treat, ameliorate, or prevent diseases or disorders (e.g. as listed in the "Preferred Indication Y" column of Table 1) which are known in the art to commonly occur in patients simultaneously, concurrently, or consecutively, or which commonly occur in patients in association with one another.

[0279] Exemplary fusion proteins of the invention containing multiple Therapeutic protein portions fused at the N- and C- termini of albumin include, but are not limited to, GCSF-HSA-EPO, EPO-HSA-GCSF, IFNalpha-HSA-IL2, IL2-HSA-IFNalpha, GCSF-HSA-IL2, IL2-HSA-GCSF, IL2-HSA-EPO, EPO-HSA-IL2, IL3-HSA-EPO, EPO-HSA-IL3, GCSF-HSA-GMCSF, GMCSF-HSA-GCSF, IL2-HSA-GMCSF, GMCSF-HSA-IL2, PTH-HSA-Calcitonin, Calcitonin-HSA-PTH, PTH-PTH-HSA-Calcitonin, Calcitonin-HSA-PTH-PTH, PTH-PTH-HSA-PTH, or PTH-HSA-PTH-PTH.

[0280] Albumin fusion proteins of the invention encompass proteins containing one, two, three, four, or more molecules of a given Therapeutic protein X or variant thereof fused to the N- or C- terminus of an albumin fusion protein of the invention, and/or to the N- and/or C- terminus of albumin or variant thereof. Molecules of a given Therapeutic protein X or variants thereof may be in any number of orientations, including, but not limited to, a 'head to head' orientation (e.g., wherein the N-terminus of one molecule of a Therapeutic protein X is fused to the N-terminus of another molecule of the Therapeutic protein X), or a 'head to tail' orientation (e.g., wherein the C-terminus of one molecule of a Therapeutic protein X is fused to the N-terminus of another molecule of Therapeutic protein X).

[0281] In one embodiment, one, two, three, or more tandemly oriented Therapeutic protein X polypeptides (or fragments or variants thereof) are fused to the N- or C- terminus of an albumin fusion protein of the invention, and/or to the N- and/or C- terminus of albumin or variant thereof.

[0282] In a specific embodiment, one, two, three, four, five, or more tandemly oriented molecules of PTH are fused to the N- or C-terminus of albumin or variant thereof. For example, one, two, three, four, five, or more tandemly oriented molecules of PTH (including, but not limited to, molecules of PTH comprising, or alternatively consisting of, amino acids 1 to 34) are fused to the N- or C-terminus of albumin or variant thereof. Exemplary fusion proteins of the invention containing multiple protein portions of PTH, include, but are not limited to, PTH-PTH-HSA, HSA-PTH-PTH, PTH-PTH-PTH-HSA, HSA-PTH-PTH-PTH, PTH-PTH-PTH-PTH-HSA, or HSA-PTH-PTH-PTH-PTH.

**[0283]** In another specific embodiment, one, two, three, four, five, or more tandemly oriented molecules of GLP-1 are fused to the N- or C-terminus of albumin or variant thereof. For example, one, two, three, four, five, or more tandemly oriented molecules of GLP-1 (including, but not limited to, molecules of GLP-1 comprising, or alternatively consisting of, amino acids 7 to 36, with residue 8 being mutated from an Alanine to a Glycine) (See for Example, the mutants disclosed in U.S. Patent No. 5,545,618, herein incorporated by reference in its entirety) are fused to the N- or C-terminus of albumin or variant thereof. Exemplary fusion proteins of the invention containing multiple protein portions of GLP-1, include, but are not limited to, GL1-GLP1-HSA, HSA-GLP1-GLP1, GLP1mutant-GLP1mutant-HSA, HSA-GLP1mutant-GLP1mutant, GLP1mutant-GLP1-HSA, HSA-GLP1mutant-GLP1, GLP1-GLP1mutant-HSA, or HSA-GLP1-GLP1mutant. Particularly preferred embodiments are GLP-1 tandem fusions such as construct ID #3070 and the protein encoded by such construct.

**[0284]** Albumin fusion proteins of the invention further encompass proteins containing one, two, three, four, or more molecules of a given Therapeutic protein X or variant thereof fused to the N- or C- terminus of an albumin fusion protein of the invention, and/or to the N- and/or C- terminus of albumin or variant thereof, wherein the molecules are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Albumin fusion proteins comprising multiple Therapeutic protein X polypeptides separated by peptide linkers may be produced using conventional recombinant DNA technology. Linkers are particularly important when fusing a small peptide to the large HSA molecule. The peptide itself can be a linker by fusing tandem copies of the peptide (see for example GLP-1) or other known linkers can be used. Constructs that incorporate linkers are described in Table 2 or are apparent when examining SEQ ID NO:Y.

**[0285]** Further, albumin fusion proteins of the invention may also be produced by fusing a Therapeutic protein X or variants thereof to the N-terminal and/or C-terminal of albumin or variants thereof in such a way as to allow the formation of intramolecular and/or intermolecular multimeric forms. In one embodiment of the invention, albumin fusion proteins may be in monomeric or multimeric forms (i.e., dimers, trimers, tetramers and higher multimers). In a further embodiment of the invention, the Therapeutic protein portion of an albumin fusion protein may be in monomeric form or multimeric form (i.e., dimers, trimers, tetramers and higher multimers). In a specific embodiment, the Therapeutic protein portion

of an albumin fusion protein is in multimeric form (i.e., dimers, trimers, tetramers and higher multimers), and the albumin protein portion is in monomeric form.

**[0286]** In addition to albumin fusion protein in which the albumin portion is fused N-terminal and/or C-terminal of the Therapeutic protein portion, albumin fusion proteins of the invention may also be produced by inserting the Therapeutic protein or peptide of interest (e.g., a Therapeutic protein X as disclosed in Table 1, or an antibody that binds a Therapeutic protein or a fragment or variant thereof) into an internal region of HA. For instance, within the protein sequence of the HA molecule a number of loops or turns exist between the end and beginning of  $\alpha$ -helices, which are stabilized by disulphide bonds. The loops, as determined from the crystal structure of HA (PDB identifiers 1AO6, 1BJ5, 1BKE, 1BM0, 1E7E to 1E7I and 1UOR) for the most part extend away from the body of the molecule. These loops are useful for the insertion, or internal fusion, of therapeutically active peptides, particularly those requiring a secondary structure to be functional, or Therapeutic proteins, to essentially generate an albumin molecule with specific biological activity.

**[0287]** Loops in human albumin structure into which peptides or polypeptides may be inserted to generate albumin fusion proteins of the invention include: Val54-Asn61, Thr76-Asp89, Ala92-Glu100, Gln170-Ala176, His 247 - Glu252, Glu 266 - Glu277, Glu 280-His288, Ala362-Glu368, Lys439-Pro447, Val462-Lys475, Thr478-Pro486, and Lys560-Thr566. In more preferred embodiments, peptides or polypeptides are inserted into the Val54-Asn61, Gln170-Ala176, and/or Lys560-Thr566 loops of mature human albumin (SEQ ID NO:1038).

**[0288]** Peptides to be inserted may be derived from either phage display or synthetic peptide libraries screened for specific biological activity or from the active portions of a molecule with the desired function. Additionally, random peptide libraries may be generated within particular loops or by insertions of randomized peptides into particular loops of the HA molecule and in which all possible combinations of amino acids are represented.

**[0289]** Such library(s) could be generated on HA or domain fragments of HA by one of the following methods:

**[0290]** randomized mutation of amino acids within one or more peptide loops of HA or HA domain fragments. Either one, more or all the residues within a loop could be mutated in this manner;

**[0291]** replacement of, or insertion into one or more loops of HA or HA domain fragments (i.e., internal fusion) of a randomized peptide(s) of length  $X_n$  (where X is an amino

acid and n is the number of residues;

[0292] N-, C- or N- and C- terminal peptide/protein fusions in addition to (a) and/or (b).

[0293] The HA or HA domain fragment may also be made multifunctional by grafting the peptides derived from different screens of different loops against different targets into the same HA or HA domain fragment.

[0294] In preferred embodiments, peptides inserted into a loop of human serum albumin are peptide fragments or peptide variants of the Therapeutic proteins disclosed in Table 1. More particularly, the invention encompasses albumin fusion proteins which comprise peptide fragments or peptide variants at least 7 at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 35, or at least 40 amino acids in length inserted into a loop of human serum albumin. The invention also encompasses albumin fusion proteins which comprise peptide fragments or peptide variants at least 7 at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 35, or at least 40 amino acids fused to the N-terminus of human serum albumin. The invention also encompasses albumin fusion proteins which comprise peptide fragments or peptide variants at least 7 at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 35, or at least 40 amino acids fused to the C-terminus of human serum albumin. For example, short peptides described in Table 1 and 2 (e.g., Therapeutic Y) can be inserted into the albumin loops.

[0295] Generally, the albumin fusion proteins of the invention may have one HA-derived region and one Therapeutic protein-derived region. Multiple regions of each protein, however, may be used to make an albumin fusion protein of the invention. Similarly, more than one Therapeutic protein may be used to make an albumin fusion protein of the invention. For instance, a Therapeutic protein may be fused to both the N- and C-terminal ends of the HA. In such a configuration, the Therapeutic protein portions may be the same or different Therapeutic protein molecules. The structure of bifunctional albumin fusion proteins may be represented as: X-HA-Y or Y-HA-X.

[0296] For example, an anti-BLyS™ scFv-HA-IFN $\alpha$ -2b fusion may be prepared to modulate the immune response to IFN $\alpha$ -2b by anti-BLyS™ scFv. An alternative is making a bi (or even multi) functional dose of HA-fusions e.g. HA-IFN $\alpha$ -2b fusion mixed with HA-anti-BLyS™ scFv fusion or other HA-fusions in various ratio's depending on function, half-



life etc.

[0297] Bi- or multi-functional albumin fusion proteins may also be prepared to target the Therapeutic protein portion of a fusion to a target organ or cell type via protein or peptide at the opposite terminus of HA.

[0298] As an alternative to the fusion of known therapeutic molecules, the peptides could be obtained by screening libraries constructed as fusions to the N-, C- or N- and C-termini of HA, or domain fragment of HA, of typically 6, 8, 12, 20 or 25 or  $X_n$  (where X is an amino acid (aa) and n equals the number of residues) randomized amino acids, and in which all possible combinations of amino acids were represented. A particular advantage of this approach is that the peptides may be selected *in situ* on the HA molecule and the properties of the peptide would therefore be as selected for rather than, potentially, modified as might be the case for a peptide derived by any other method then being attached to HA.

[0299] Additionally, the albumin fusion proteins of the invention may include a linker peptide between the fused portions to provide greater physical separation between the moieties and thus maximize the accessibility of the Therapeutic protein portion, for instance, for binding to its cognate receptor. The linker peptide may consist of amino acids such that it is flexible or more rigid.

[0300] The linker sequence may be cleavable by a protease or chemically to yield the growth hormone related moiety. Preferably, the protease is one which is produced naturally by the host, for example the *S. cerevisiae* protease *kex2* or equivalent proteases.

[0301] Therefore, as described above, the albumin fusion proteins of the invention may have the following formula R1-L-R2; R2-L-R1; or R1-L-R2-L-R1, wherein R1 is at least one Therapeutic protein, peptide or polypeptide sequence, and not necessarily the same Therapeutic protein, L is a linker and R2 is a serum albumin sequence.

[0302] In preferred embodiments, Albumin fusion proteins of the invention comprising a Therapeutic protein have extended shelf life compared to the shelf life the same Therapeutic protein when not fused to albumin. Shelf-life typically refers to the time period over which the therapeutic activity of a Therapeutic protein in solution or in some other storage formulation, is stable without undue loss of therapeutic activity. Many of the Therapeutic proteins are highly labile in their unfused state. As described below, the typical shelf-life of these Therapeutic proteins is markedly prolonged upon incorporation into the albumin fusion protein of the invention.

[0303] Albumin fusion proteins of the invention with “prolonged” or “extended”

shelf-life exhibit greater therapeutic activity relative to a standard that has been subjected to the same storage and handling conditions. The standard may be the unfused full-length Therapeutic protein. When the Therapeutic protein portion of the albumin fusion protein is an analog, a variant, or is otherwise altered or does not include the complete sequence for that protein, the prolongation of therapeutic activity may alternatively be compared to the unfused equivalent of that analog, variant, altered peptide or incomplete sequence. As an example, an albumin fusion protein of the invention may retain greater than about 100% of the therapeutic activity, or greater than about 105%, 110%, 120%, 130%, 150% or 200% of the therapeutic activity of a standard when subjected to the same storage and handling conditions as the standard when compared at a given time point.

**[0304]** Shelf-life may also be assessed in terms of therapeutic activity remaining after storage, normalized to therapeutic activity when storage began. Albumin fusion proteins of the invention with prolonged or extended shelf-life as exhibited by prolonged or extended therapeutic activity may retain greater than about 50% of the therapeutic activity, about 60%, 70%, 80%, or 90% or more of the therapeutic activity of the equivalent unfused Therapeutic protein when subjected to the same conditions. For example, as discussed in Example 38, an albumin fusion protein of the invention comprising hGH fused to the full length HA sequence may retain about 80% or more of its original activity in solution for periods of up to 5 weeks or more under various temperature conditions.

### ***Expression of Fusion Proteins***

**[0305]** The albumin fusion proteins of the invention may be produced as recombinant molecules by secretion from yeast, a microorganism such as a bacterium, or a human or animal cell line. Preferably, the polypeptide is secreted from the host cells.

**[0306]** A particular embodiment of the invention comprises a DNA construct encoding a signal sequence effective for directing secretion in yeast, particularly a yeast-derived signal sequence (especially one which is homologous to the yeast host), and the fused molecule of the first aspect of the invention, there being no yeast-derived pro sequence between the signal and the mature polypeptide.

**[0307]** The *Saccharomyces cerevisiae* invertase signal is a preferred example of a yeast-derived signal sequence.

**[0308]** Conjugates of the kind prepared by Poznansky *et al.*, (FEBS Lett. 239:18 (1988)), in which separately-prepared polypeptides are joined by chemical cross-linking, are

not contemplated.

**[0309]** The present invention also includes a cell, preferably a yeast cell transformed to express an albumin fusion protein of the invention. In addition to the transformed host cells themselves, the present invention also contemplates a culture of those cells, preferably a monoclonal (clonally homogeneous) culture, or a culture derived from a monoclonal culture, in a nutrient medium. If the polypeptide is secreted, the medium will contain the polypeptide, with the cells, or without the cells if they have been filtered or centrifuged away. Many expression systems are known and may be used, including bacteria (for example *E. coli* and *Bacillus subtilis*), yeasts (for example *Saccharomyces cerevisiae*, *Kluyveromyces lactis* and *Pichia pastoris*, filamentous fungi (for example *Aspergillus*), plant cells, animal cells and insect cells.

**[0310]** Preferred yeast strains to be used in the production of albumin fusion proteins are D88, DXY1 and BXP10. D88 [*leu2-3*, *leu2-122*, *can1*, *pral*, *ubc4*] is a derivative of parent strain AH22<sup>his</sup><sup>+</sup> (also known as DB1; see, e.g., Sleep *et al.* Biotechnology 8:42-46 (1990)). The strain contains a *leu2* mutation which allows for auxotrophic selection of 2 micron-based plasmids that contain the LEU2 gene. D88 also exhibits a derepression of PRB1 in glucose excess. The PRB1 promoter is normally controlled by two checkpoints that monitor glucose levels and growth stage. The promoter is activated in wild type yeast upon glucose depletion and entry into stationary phase. Strain D88 exhibits the repression by glucose but maintains the induction upon entry into stationary phase. The PRA1 gene encodes a yeast vacuolar protease, YscA endoprotease A, that is localized in the ER. The UBC4 gene is in the ubiquitination pathway and is involved in targeting short lived and abnormal proteins for ubiquitin dependant degradation. Isolation of this *ubc4* mutation was found to increase the copy number of an expression plasmid in the cell and cause an increased level of expression of a desired protein expressed from the plasmid (see, e.g., International Publication No. WO99/00504, hereby incorporated in its entirety by reference herein).

**[0311]** DXY1, a derivative of D88, has the following genotype: [*leu2-3*, *leu2-122*, *can1*, *pral*, *ubc4*, *ura3::yap3*]. In addition to the mutations isolated in D88, this strain also has a knockout of the YAP3 protease. This protease causes cleavage of mostly di-basic residues (RR, RK, KR, KK) but can also promote cleavage at single basic residues in proteins. Isolation of this *yap3* mutation resulted in higher levels of full length HSA production (see, e.g., U.S. Patent No. 5,965,386 and Kerry-Williams *et al.*, Yeast 14:161-169 (1998), hereby incorporated in their entireties by reference herein).

[0312] BXP10 has the following genotype: *leu2-3, leu2-122, can1, pral, ubc4, ura3, yap3::URA3, lys2, hsp150::LYS2, pmt1::URA3*. In addition to the mutations isolated in DXY1, this strain also has a knockout of the PMT1 gene and the HSP150 gene. The PMT1 gene is a member of the evolutionarily conserved family of dolichyl-phosphate-D-mannose protein O-mannosyltransferases (Pmts). The transmembrane topology of Pmt1p suggests that it is an integral membrane protein of the endoplasmic reticulum with a role in O-linked glycosylation. This mutation serves to reduce/eliminate O-linked glycosylation of HSA fusions (see, e.g., International Publication No. WO00/44772, hereby incorporated in its entirety by reference herein). Studies revealed that the Hsp150 protein is inefficiently separated from rHA by ion exchange chromatography. The mutation in the HSP150 gene removes a potential contaminant that has proven difficult to remove by standard purification techniques. See, e.g., U.S. Patent No. 5,783,423, hereby incorporated in its entirety by reference herein.

[0313] The desired protein is produced in conventional ways, for example from a coding sequence inserted in the host chromosome or on a free plasmid. The yeasts are transformed with a coding sequence for the desired protein in any of the usual ways, for example electroporation. Methods for transformation of yeast by electroporation are disclosed in Becker & Guarente (1990) *Methods Enzymol.* 194, 182.

[0314] Successfully transformed cells, *i.e.*, cells that contain a DNA construct of the present invention, can be identified by well known techniques. For example, cells resulting from the introduction of an expression construct can be grown to produce the desired polypeptide. Cells can be harvested and lysed and their DNA content examined for the presence of the DNA using a method such as that described by Southern (1975) *J. Mol. Biol.* 98, 503 or Berent *et al.* (1985) *Biotech.* 3, 208. Alternatively, the presence of the protein in the supernatant can be detected using antibodies.

[0315] Useful yeast plasmid vectors include pRS403-406 and pRS413-416 and are generally available from Stratagene Cloning Systems, La Jolla, CA 92037, USA. Plasmids pRS403, pRS404, pRS405 and pRS406 are Yeast Integrating plasmids (YIps) and incorporate the yeast selectable markers HIS3, 7RP1, LEU2 and URA3. Plasmids pRS413-416 are Yeast Centromere plasmids (Ycps).

[0316] Preferred vectors for making albumin fusion proteins for expression in yeast include pPPC0005, pScCHSA, pScNHSA, and pC4:HSA which are described in detail in Example 1. Figure 2 shows a map of the pPPC0005 plasmid that can be used as the base

vector into which polynucleotides encoding Therapeutic proteins may be cloned to form HA-fusions. It contains a *PRB1 S. cerevisiae* promoter (PRB1p), a Fusion leader sequence (FL), DNA encoding HA (rHA) and an *ADH1 S. cerevisiae* terminator sequence. The sequence of the fusion leader sequence consists of the first 19 amino acids of the signal peptide of human serum albumin (SEQ ID NO:1094) and the last five amino acids of the mating factor alpha 1 promoter (SLDKR, see EP-A-387 319 which is hereby incorporated by reference in its entirety).

[0317] The plasmids, pPPC0005, pScCHSA, pScNHSA, and pC4:HSA were deposited on April 11, 2001 at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209 and given accession numbers ATCC PTA-3278, PTA-3276, PTA-3279, and PTA-3277, respectively. Another vector useful for expressing an albumin fusion protein in yeast the pSAC35 vector which is described in Sleep *et al.*, BioTechnology 8:42 (1990) which is hereby incorporated by reference in its entirety.

[0318] Another yeast promoter that can be used to express the albumin fusion protein is the MET25 promoter. See, for example, Dominik Mumburg, Rolf Muller and Martin Funk. Nucleic Acids Research, 1994, Vol. 22, No. 25, pp. 5767-5768. The Met25 promoter is 383 bases long (bases -382 to -1) and the genes expressed by this promoter are also known as Met15, Met17, and YLR303W. A preferred embodiment uses the sequence below, where, at the 5' end of the sequence below, the Not 1 site used in the cloning is underlined and at the 3' end, the ATG start codon is underlined:

GCGGCCCGCCGGATGCAAGGGTTCGAATCCCTTAGCTCTCATTATTTTTTGGCTTTTT  
CTCTTGAGGTCACATGATCGCAAATGGCAAATGGCACGTGAAGCTGTCGATATT  
GGGGAAGTGTGGTGGTTGGCAAATGACTAATTAAGTTAGTCAAGGCGCCATCCTC  
ATGAAAGTGTGTAACATAATAACCGAAGTGTCGAAAAGGTGGCACCTTGTCCA  
ATTGAACACGCTCGATGAAAAAATAAGATATATATAAGGTAAAGTAAAGCGTC  
TGTTAGAAAGGAAGTTTTTCCTTTTTCTTGCTCTCTTGTCTTTTCATCTACTATTTT  
CTTCGTGTAATACAGGGTCGTCAGATACATAGATACAATTCTATTACCCCCATCC  
ATACAATG (SEQ ID NO: 2138)

[0319] A variety of methods have been developed to operably link DNA to vectors via complementary cohesive termini. For instance, complementary homopolymer tracts can be added to the DNA segment to be inserted to the vector DNA. The vector and DNA segment are then joined by hydrogen bonding between the complementary homopolymeric tails to form recombinant DNA molecules.

[0320] Synthetic linkers containing one or more restriction sites provide an alternative method of joining the DNA segment to vectors. The DNA segment, generated by endonuclease restriction digestion, is treated with bacteriophage T4 DNA polymerase or E. coli DNA polymerase I, enzymes that remove protruding, gamma-single-stranded termini with their 3' 5'-exonucleolytic activities, and fill in recessed 3'-ends with their polymerizing activities.

[0321] The combination of these activities therefore generates blunt-ended DNA segments. The blunt-ended segments are then incubated with a large molar excess of linker molecules in the presence of an enzyme that is able to catalyze the ligation of blunt-ended DNA molecules, such as bacteriophage T4 DNA ligase. Thus, the products of the reaction are DNA segments carrying polymeric linker sequences at their ends. These DNA segments are then cleaved with the appropriate restriction enzyme and ligated to an expression vector that has been cleaved with an enzyme that produces termini compatible with those of the DNA segment.

[0322] Synthetic linkers containing a variety of restriction endonuclease sites are commercially available from a number of sources including International Biotechnologies Inc, New Haven, CT, USA.

[0323] A desirable way to modify the DNA in accordance with the invention, if, for example, HA variants are to be prepared, is to use the polymerase chain reaction as disclosed by Saiki *et al.* (1988) *Science* 239, 487-491. In this method the DNA to be enzymatically amplified is flanked by two specific oligonucleotide primers which themselves become incorporated into the amplified DNA. The specific primers may contain restriction endonuclease recognition sites which can be used for cloning into expression vectors using methods known in the art.

[0324] Exemplary genera of yeast contemplated to be useful in the practice of the present invention as hosts for expressing the albumin fusion proteins are *Pichia* (Hansenula), *Saccharomyces*, *Kluyveromyces*, *Candida*, *Torulopsis*, *Torulaspora*, *Schizosaccharomyces*, *Citeromyces*, *Pachysolen*, *Debaromyces*, *Metschunikowia*, *Rhodosporidium*, *Leucosporidium*, *Botryoascus*, *Sporidiobolus*, *Endomycopsis*, and the like. Preferred genera are those selected from the group consisting of *Saccharomyces*, *Schizosaccharomyces*, *Kluyveromyces*, *Pichia* and *Torulaspora*. Examples of *Saccharomyces* spp. are *S. cerevisiae*, *S. italicus* and *S. rouxii*.

[0325] Examples of *Kluyveromyces* spp. are *K. fragilis*, *K. lactis* and *K. marxianus*. A suitable *Torulaspora* species is *T. delbrueckii*. Examples of *Pichia* (Hansenula) spp. are *P.*

*angusta* (formerly *H. polymorpha*), *P. anomala* (formerly *H. anomala*) and *P. pastoris*. Methods for the transformation of *S. cerevisiae* are taught generally in EP 251 744, EP 258 067 and WO 90/01063, all of which are incorporated herein by reference.

[0326] Preferred exemplary species of *Saccharomyces* include *S. cerevisiae*, *S. italicus*, *S. diastaticus*, and *Zygosaccharomyces rouxii*. Preferred exemplary species of *Kluyveromyces* include *K. fragilis* and *K. lactis*. Preferred exemplary species of *Hansenula* include *H. polymorpha* (now *Pichia angusta*), *H. anomala* (now *Pichia anomala*), and *Pichia capsulata*. Additional preferred exemplary species of *Pichia* include *P. pastoris*. Preferred exemplary species of *Aspergillus* include *A. niger* and *A. nidulans*. Preferred exemplary species of *Yarrowia* include *Y. lipolytica*. Many preferred yeast species are available from the ATCC. For example, the following preferred yeast species are available from the ATCC and are useful in the expression of albumin fusion proteins: *Saccharomyces cerevisiae* Hansen, teleomorph strain BY4743 *yap3* mutant (ATCC Accession No. 4022731); *Saccharomyces cerevisiae* Hansen, teleomorph strain BY4743 *hsp150* mutant (ATCC Accession No. 4021266); *Saccharomyces cerevisiae* Hansen, teleomorph strain BY4743 *pmt1* mutant (ATCC Accession No. 4023792); *Saccharomyces cerevisiae* Hansen, teleomorph (ATCC Accession Nos. 20626; 44773; 44774; and 62995); *Saccharomyces diastaticus* Andrews et Gilliland ex van der Walt, teleomorph (ATCC Accession No. 62987); *Kluyveromyces lactis* (Dombrowski) van der Walt, teleomorph (ATCC Accession No. 76492); *Pichia angusta* (Teunisson et al.) Kurtzman, teleomorph deposited as *Hansenula polymorpha* de Morais et Maia, teleomorph (ATCC Accession No. 26012); *Aspergillus niger* van Tieghem, anamorph (ATCC Accession No. 9029); *Aspergillus niger* van Tieghem, anamorph (ATCC Accession No. 16404); *Aspergillus nidulans* (Eidam) Winter, anamorph (ATCC Accession No. 48756); and *Yarrowia lipolytica* (Wickerham et al.) van der Walt et von Arx, teleomorph (ATCC Accession No. 201847).

[0327] Suitable promoters for *S. cerevisiae* include those associated with the PGKI gene, GAL1 or GAL10 genes, CYCI, PHO5, TRPI, ADHI, ADH2, the genes for glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, triose phosphate isomerase, phosphoglucose isomerase, glucokinase, alpha-mating factor pheromone, [a mating factor pheromone], the PRBI promoter, the GUT2 promoter, the GPDI promoter, and hybrid promoters involving hybrids of parts of 5' regulatory regions with parts of 5' regulatory regions of other promoters or with upstream activation sites (e.g. the promoter of EP-A-258 067).

[0328] Convenient regulatable promoters for use in *Schizosaccharomyces pombe* are the thiamine-repressible promoter from the nmt gene as described by Maundrell (1990) *J. Biol. Chem.* 265, 10857-10864 and the glucose repressible jbp1 gene promoter as described by Hoffman & Winston (1990) *Genetics* 124, 807-816.

[0329] Methods of transforming *Pichia* for expression of foreign genes are taught in, for example, Cregg *et al.* (1993), and various Phillips patents (*e.g.* US 4 857 467, incorporated herein by reference), and *Pichia* expression kits are commercially available from Invitrogen BV, Leek, Netherlands, and Invitrogen Corp., San Diego, California. Suitable promoters include AOX1 and AOX2. Gleeson *et al.* (1986) *J. Gen. Microbiol.* 132, 3459-3465 include information on *Hansenula* vectors and transformation, suitable promoters being MOX1 and FMD1; whilst EP 361 991, Fleer *et al.* (1991) and other- publications from Rhone-Poulenc Rorer teach how to express foreign proteins in *Kluyveromyces* spp., a suitable promoter being PGKI.

[0330] The transcription termination signal is preferably the 3' flanking sequence of a eukaryotic gene which contains proper signals for transcription termination and polyadenylation. Suitable 3' flanking sequences may, for example, be those of the gene naturally linked to the expression control sequence used, *i.e.* may correspond to the promoter. Alternatively, they may be different in which case the termination signal of the *S. cerevisiae* ADHI gene is preferred.

[0331] The desired albumin fusion protein may be initially expressed with a secretion leader sequence, which may be any leader effective in the yeast chosen. Leaders useful in yeast include any of the following:

- a) the MPIF-1 signal sequence (*e.g.*, amino acids 1-21 of GenBank Accession number AAB51134) MKVSVAALSCLMLVTALGSQA (SEQ ID NO:2132)
- b) the stanniocalcin signal sequence (MLQNSAVLLLLVISASA, SEQ ID NO:1054)
- c) the pre-pro region of the HSA signal sequence (*e.g.*, MKWVTFISLLFLFSSAYS SRGVFRR, SEQ ID NO:1176)
- d) the pre region of the HSA signal sequence (*e.g.*, MKWVTFISLLFLFSSAYS, SEQ ID NO:1177) or variants thereof, such as, for example, MKWVSFISLLFLFSSAYS, (SEQ ID NO:1168)
- e) the invertase signal sequence (*e.g.*, MLLQAFLFLAGFAAKISA, SEQ ID NO:1108)
- f) the yeast mating factor alpha signal sequence (*e.g.*,



MRFPSIFTAVLAFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDV  
 AVLPFNSTNNGLLFINTTIAAIAAKEEGVSLEKR, SEQ ID NO:1109 or  
 MRFPSIFTAVLAFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDV  
 AVLPFNSTNNGLLFINTTIAAIAAKEEGVSLDKR, SEQ ID NO:1109)

- g) *K. lactis* killer toxin leader sequence
- h) a hybrid signal sequence (e.g., MKWVSFISLLFLFSSAYSRSLEKR, SEQ ID NO:1110)
- i) an HSA/MF $\alpha$ -1 hybrid signal sequence (also known as HSA/kex2) (e.g., MKWVSFISLLFLFSSAYSRSLEDKR, SEQ ID NO:1111)
- j) a *K. lactis* killer/ MF $\alpha$ -1 fusion leader sequence (e.g., MNIFYIFLFLSFVQGSLEDKR, SEQ ID NO:1169)
- k) the Immunoglobulin Ig signal sequence (e.g., MGWSCILFLVATATGVHS, SEQ ID NO:1095)
- l) the Fibulin B precursor signal sequence (e.g., MERAAPSRRVPLPLLLGGLALLAAGVDA, SEQ ID NO:1096)
- m) the clusterin precursor signal sequence (e.g., MMKTLLLFVGLLLTWESGQVLG, SEQ ID NO:1097)
- n) the insulin-like growth factor-binding protein 4 signal sequence (e.g., MLPLCLVAALLAAGPGPSLG, SEQ ID NO:1098)
- o) variants of the pre-pro-region of the HSA signal sequence such as, for example, MKWVSFISLLFLFSSAYSRGVFRR (SEQ ID NO:1167), MKWVTFISLLFLFAGVLG (SEQ ID NO:1099), MKWVTFISLLFLFSGVLG (SEQ ID NO:1100), MKWVTFISLLFLFGGVLG (SEQ ID NO:1101),  
 Modified HSA leader HSA #64  
 MKWVTFISLLFLFAGVSG (SEQ ID NO:2133);  
 Modified HSA leader HSA #66  
 MKWVTFISLLFLFGGVSG (SEQ ID NO:2134);  
 Modified HSA (A14) leader –  
 MKWVTFISLLFLFAGVSG (SEQ ID NO: 1102);  
 Modified HSA (S14) leader (also known as modified HSA #65) –  
 MKWVTFISLLFLFSGVSG (SEQ ID NO:1103),  
 Modified HSA (G14) leader –

- MKWVTFISLLFLFGGVSG (SEQ ID NO:1104), or  
MKWVTFISLLFLFGGVLGDLHKS (SEQ ID NO:1105)
- p) a consensus signal sequence (MPTWAWWLFLVLLLALWAPARG, SEQ ID NO:1055)
  - q) acid phosphatase (PH05) leader (e.g., MFKSVVYSILAASLANA SEQ ID NO:2135)
  - r) the pre-sequence of MFoz-1
  - s) the pre-sequence of 0 glucanase (BGL2)
  - t) killer toxin leader
  - u) the presequence of killer toxin
  - v) k. lactis killer toxin prepro (29 amino acids; 16 amino acids of pre and 13 amino acids of pro) MNIFYIFLFLLSFVQGLEHTHRRGSLDKR (SEQ ID NO:2136)
  - w) *S. diastaticus* glucoamylase II secretion leader sequence
  - x) *S. carlsbergensis*  $\alpha$ -galactosidase (MEL1) secretion leader sequence
  - y) *Candida glucoamylase* leader sequence
  - z) The hybrid leaders disclosed in EP-A-387 319 (herin incorporated by reference)
  - aa) the gp67 signal sequence (in conjunction with baculoviral expression systems) (e.g., amino acids 1-19 of GenBank Accession Number AAA72759) or
  - bb) the natural leader of the therapeutic protein X;
  - cc) *S. cerevisiae* invertase (SUC2) leader, as disclosed in JP 62-096086 (granted as 911036516, herein incorporate by reference); or
  - dd) Inulinase – MKLAYSLLLPLAGVSASVINYKR (SEQ ID NO:2137).
  - ee) A modified TA57 propeptide leader variant #1 –  
MKLKTVRSAVLSSLFASQVLGQPIDDTESQTTSVNLMADDTESAFATQTN  
SGGLDVVGLISMAKR (SEQ ID NO:2128)
  - ff) A modified TA57 propeptide leader variant #2 –  
MKLKTVRSAVLSSLFASQVLGQPIDDTESQTTSVNLMADDTESAFATQTN  
SGGLDVVGLISMAEEGEPKR (SEQ ID NO:2129)

### **Additional Methods of Recombinant and Synthetic Production of Albumin Fusion Proteins**

[0332] The present invention also relates to vectors containing a polynucleotide encoding an albumin fusion protein of the present invention, host cells, and the production of

albumin fusion proteins by synthetic and recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

[0333] The polynucleotides encoding albumin fusion proteins of the invention may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

[0334] The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli lac*, *trp*, *phoA* and *tac* promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

[0335] As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418, glutamine synthase, or neomycin resistance for eukaryotic cell culture, and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells (e.g., *Saccharomyces cerevisiae* or *Pichia pastoris* (ATCC Accession No. 201178)); insect cells such as *Drosophila* S2 and *Spodoptera Sf9* cells; animal cells such as CHO, COS, NSO, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

[0336] Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from

Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlsbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

[0337] In one embodiment, polynucleotides encoding an albumin fusion protein of the invention may be fused to signal sequences which will direct the localization of a protein of the invention to particular compartments of a prokaryotic or eukaryotic cell and/or direct the secretion of a protein of the invention from a prokaryotic or eukaryotic cell. For example, in *E. coli*, one may wish to direct the expression of the protein to the periplasmic space. Examples of signal sequences or proteins (or fragments thereof) to which the albumin fusion proteins of the invention may be fused in order to direct the expression of the polypeptide to the periplasmic space of bacteria include, but are not limited to, the *pelB* signal sequence, the maltose binding protein (MBP) signal sequence, MBP, the *ompA* signal sequence, the signal sequence of the periplasmic *E. coli* heat-labile enterotoxin B-subunit, and the signal sequence of alkaline phosphatase. Several vectors are commercially available for the construction of fusion proteins which will direct the localization of a protein, such as the pMAL series of vectors (particularly the pMAL-p series) available from New England Biolabs. In a specific embodiment, polynucleotides albumin fusion proteins of the invention may be fused to the *pelB* pectate lyase signal sequence to increase the efficiency of expression and purification of such polypeptides in Gram-negative bacteria. See, U.S. Patent Nos. 5,576,195 and 5,846,818, the contents of which are herein incorporated by reference in their entireties.

[0338] Examples of signal peptides that may be fused to an albumin fusion protein of the invention in order to direct its secretion in mammalian cells include, but are not limited to:

- a) the MPIF-1 signal sequence (e.g., amino acids 1-21 of GenBank Accession number AAB51134) MKVSVAALSCLMLVTALGSQA (SEQ ID NO:2132)
- b) the stanniocalcin signal sequence (MLQNSAVLLLLVISASA, SEQ ID NO:1054)
- c) the pre-pro region of the HSA signal sequence (e.g., MKWVTFISLLFLFSSAYS RGVFRR, SEQ ID NO:1176)
- d) the pre region of the HSA signal sequence (e.g., MKWVTFISLLFLFSSAYS, SEQ ID NO:1177) or variants thereof, such as, for example, MKWVSFISLLFLFSSAYS, (SEQ ID NO:1168)

- e) the invertase signal sequence (e.g., MLLQAFLFLAGFAAKISA, SEQ ID NO:1108)
- f) the yeast mating factor alpha signal sequence (e.g.,  
MRFPSIFTAVLAFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVL  
PFSNSTNNGLLFINTTASIAAKEEGVSLEKR, SEQ ID NO:1109 or  
MRFPSIFTAVLAFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVL  
PFSNSTNNGLLFINTTASIAAKEEGVSLDKR, SEQ ID NO:1109)
- g) *K. lactis* killer toxin leader sequence
- h) a hybrid signal sequence (e.g., MKWVSFISLLFLFSSAYSRSLEKR, SEQ ID NO:1110)
- i) an HSA/MF $\alpha$ -1 hybrid signal sequence (also known as HSA/kex2) (e.g.,  
MKWVSFISLLFLFSSAYSRSLEDKR, SEQ ID NO:1111)
- j) a *K. lactis* killer/ MF $\alpha$ -1 fusion leader sequence (e.g.,  
MNIFYIFLFLSFVQGSLEDKR, SEQ ID NO:1169)
- k) the Immunoglobulin Ig signal sequence (e.g., MGWSCILFLVATATGVHS, SEQ ID NO:1095)
- l) the Fibulin B precursor signal sequence (e.g.,  
MERAAPSRRVPLPLLLLGLALLAAGVDA, SEQ ID NO:1096)
- m) the clusterin precursor signal sequence (e.g.,  
MMKTLLLFVGLLLTWESGQVLG, SEQ ID NO:1097)
- n) the insulin-like growth factor-binding protein 4 signal sequence (e.g.,  
MLPLCLVAALLAAGPGPSLG, SEQ ID NO:1098)
- o) variants of the pre-pro-region of the HSA signal sequence such as, for example,  
MKWVSFISLLFLFSSAYSRGVFRR (SEQ ID NO:1167),  
MKWVTFISLLFLFAGVLG (SEQ ID NO:1099),  
MKWVTFISLLFLFSGVLG (SEQ ID NO:1100),  
MKWVTFISLLFLFGGVLG (SEQ ID NO:1101),  
Modified HSA leader HSA #64  
MKWVTFISLLFLFAGVSG (SEQ ID NO:2133);  
Modified HSA leader HSA #66  
MKWVTFISLLFLFGGVSG (SEQ ID NO:2134);  
Modified HSA (A14) leader –  
MKWVTFISLLFLFAGVSG (SEQ ID NO: 1102);

- Modified HSA (S14) leader (also known as modified HSA #65) –  
 MKWVTFISLLFLFSGVSG (SEQ ID NO:1103),
- Modified HSA (G14) leader –  
 MKWVTFISLLFLFGGVSG (SEQ ID NO:1104), or  
 MKWVTFISLLFLFGGVLDLHKS (SEQ ID NO:1105)
- p) a consensus signal sequence (MPTWAWWLFLVLLLALWAPARG, SEQ ID NO:1055)
- q) acid phosphatase (PH05) leader (e.g., MFKSVVYSILAASLANA SEQ ID NO:2135)
- r) the pre-sequence of MFoz-1
- s) the pre-sequence of 0 glucanase (BGL2)
- t) killer toxin leader
- u) the presequence of killer toxin
- v) k. lactis killer toxin prepro (29 amino acids; 16 amino acids of pre and 13 amino acids of pro) MNIFYIFLFLLSFVQGLEHTHRRGSLDKR (SEQ ID NO:2136)
- w) *S. diastaticus* glucoamylase II secretion leader sequence
- x) *S. carlsbergensis*  $\alpha$ -galactosidase (MEL1) secretion leader sequence
- y) *Candida glucoamylase* leader sequence
- z) The hybrid leaders disclosed in EP-A-387 319 (herin incorporated by reference)
- aa) the gp67 signal sequence (in conjunction with baculoviral expression systems) (e.g., amino acids 1-19 of GenBank Accession Number AAA72759) or
- bb) the natural leader of the therapeutic protein X;
- cc) *S. cerevisiae* invertase (SUC2) leader, as disclosed in JP 62-096086 (granted as 911036516, herein incorporate by reference); or
- dd) Inulinase – MKLAYSLLLPLAGVSASVINYKR (SEQ ID NO:2137).
- ee) A modified TA57 propeptide leader variant #1 –  
 MKLKTVRSAVLSSLFASQVLGQPIDDTESQTTSVNLMADDTESAFATQTNSGG  
 LDVVGLISMAKR (SEQ ID NO:2128)
- ff) A modified TA57 propeptide leader variant #2 –  
 MKLKTVRSAVLSSLFASQVLGQPIDDTESQTTSVNLMADDTESAFATQTNSG  
 GLDVVGLISMAEEGEPKR (SEQ ID NO:2129)

**[0339]** Vectors which use glutamine synthase (GS) or DHFR as the selectable markers can be amplified in the presence of the drugs methionine sulfoximine or methotrexate, respectively. An advantage of glutamine synthase based vectors are the availability of cell lines (e.g., the murine myeloma cell line, NSO) which are glutamine synthase negative. Glutamine synthase expression systems can also function in glutamine synthase expressing cells (e.g., Chinese Hamster Ovary (CHO) cells) by providing additional inhibitor to prevent the functioning of the endogenous gene. A glutamine synthase expression system and components thereof are detailed in PCT publications: WO87/04462; WO86/05807; WO89/01036; WO89/10404; and WO91/06657, which are hereby incorporated in their entireties by reference herein. Additionally, glutamine synthase expression vectors can be obtained from Lonza Biologics, Inc. (Portsmouth, NH). Expression and production of monoclonal antibodies using a GS expression system in murine myeloma cells is described in Bebbington *et al.*, *Bio/technology* 10:169(1992) and in Biblia and Robinson *Biotechnol. Prog.* 11:1 (1995) which are herein incorporated by reference.

**[0340]** The present invention also relates to host cells containing the above-described vector constructs described herein, and additionally encompasses host cells containing nucleotide sequences of the invention that are operably associated with one or more heterologous control regions (e.g., promoter and/or enhancer) using techniques known of in the art. The host cell can be a higher eukaryotic cell, such as a mammalian cell (e.g., a human derived cell), or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. A host strain may be chosen which modulates the expression of the inserted gene sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus expression of the genetically engineered polypeptide may be controlled. Furthermore, different host cells have characteristics and specific mechanisms for the translational and post-translational processing and modification (e.g., phosphorylation, cleavage) of proteins. Appropriate cell lines can be chosen to ensure the desired modifications and processing of the foreign protein expressed.

**[0341]** Introduction of the nucleic acids and nucleic acid constructs of the invention into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis *et al.*, *Basic Methods In Molecular Biology* (1986). It is specifically contemplated that

the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

[0342] In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., the coding sequence corresponding to a Therapeutic protein may be replaced with an albumin fusion protein corresponding to the Therapeutic protein), and/or to include genetic material (e.g., heterologous polynucleotide sequences such as for example, an albumin fusion protein of the invention corresponding to the Therapeutic protein may be included). The genetic material operably associated with the endogenous polynucleotide may activate, alter, and/or amplify endogenous polynucleotides.

[0343] In addition, techniques known in the art may be used to operably associate heterologous polynucleotides (e.g., polynucleotides encoding an albumin protein, or a fragment or variant thereof) and/or heterologous control regions (e.g., promoter and/or enhancer) with endogenous polynucleotide sequences encoding a Therapeutic protein via homologous recombination (see, e.g., US Patent Number 5,641,670, issued June 24, 1997; International Publication Number WO 96/29411; International Publication Number WO 94/12650; Koller *et al.*, *Proc. Natl. Acad. Sci. USA* 86:8932-8935 (1989); and Zijlstra *et al.*, *Nature* 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

[0344] Albumin fusion proteins of the invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, hydrophobic charge interaction chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

[0345] In preferred embodiments the albumin fusion proteins of the invention are purified using Anion Exchange Chromatography including, but not limited to, chromatography on Q-sepharose, DEAE sepharose, poros HQ, poros DEAE, Toyopearl Q, Toyopearl QAE, Toyopearl DEAE, Resource/Source Q and DEAE, Fractogel Q and DEAE columns.

[0346] In specific embodiments the albumin fusion proteins of the invention are



purified using Cation Exchange Chromatography including, but not limited to, SP-sepharose, CM sepharose, poros HS, poros CM, Toyopearl SP, Toyopearl CM, Resource/Source S and CM, Fractogel S and CM columns and their equivalents and comparables.

**[0347]** In specific embodiments the albumin fusion proteins of the invention are purified using Hydrophobic Interaction Chromatography including, but not limited to, Phenyl, Butyl, Methyl, Octyl, Hexyl-sepharose, poros Phenyl, Butyl, Methyl, Octyl, Hexyl, Toyopearl Phenyl, Butyl, Methyl, Octyl, Hexyl Resource/Source Phenyl, Butyl, Methyl, Octyl, Hexyl, Fractogel Phenyl, Butyl, Methyl, Octyl, Hexyl columns and their equivalents and comparables.

**[0348]** In specific embodiments the albumin fusion proteins of the invention are purified using Size Exclusion Chromatography including, but not limited to, sepharose S100, S200, S300, superdex resin columns and their equivalents and comparables.

**[0349]** In specific embodiments the albumin fusion proteins of the invention are purified using Affinity Chromatography including, but not limited to, Mimetic Dye affinity, peptide affinity and antibody affinity columns that are selective for either the HSA or the "fusion target" molecules.

**[0350]** In preferred embodiments albumin fusion proteins of the invention are purified using one or more Chromatography methods listed above. In other preferred embodiments, albumin fusion proteins of the invention are purified using one or more of the following Chromatography columns, Q sepharose FF column, SP Sepharose FF column, Q Sepharose High Performance Column, Blue Sepharose FF column, Blue Column, Phenyl Sepharose FF column, DEAE Sepharose FF, or Methyl Column.

**[0351]** Additionally, albumin fusion proteins of the invention may be purified using the process described in PCT International Publication WO 00/44772 which is herein incorporated by reference in its entirety. One of skill in the art could easily modify the process described therein for use in the purification of albumin fusion proteins of the invention.

**[0352]** Albumin fusion proteins of the present invention may be recovered from: products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, albumin fusion proteins of the invention may also include

an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

[0353] In one embodiment, the yeast *Pichia pastoris* is used to express albumin fusion proteins of the invention in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A main step in the methanol metabolization pathway is the oxidation of methanol to formaldehyde using O<sub>2</sub>. This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for O<sub>2</sub>. Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (*AOX1*) is highly active. In the presence of methanol, alcohol oxidase produced from the *AOX1* gene comprises up to approximately 30% of the total soluble protein in *Pichia pastoris*. See Ellis, S.B., *et al.*, *Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J., *et al.*, *Yeast* 5:167-77 (1989); Tschopp, J.F., *et al.*, *Nucl. Acids Res.* 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, under the transcriptional regulation of all or part of the *AOX1* regulatory sequence is expressed at exceptionally high levels in *Pichia* yeast grown in the presence of methanol.

[0354] In one example, the plasmid vector pPIC9K is used to express DNA encoding an albumin fusion protein of the invention, as set forth herein, in a *Pichea* yeast system essentially as described in "*Pichia* Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a polypeptide of the invention by virtue of the strong *AOX1* promoter linked to the *Pichia pastoris* alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

[0355] Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2,

pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

**[0356]** In another embodiment, high-level expression of a heterologous coding sequence, such as, for example, a polynucleotide encoding an albumin fusion protein of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

**[0357]** In addition, albumin fusion proteins of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, *Proteins: Structures and Molecular Principles*, W.H. Freeman & Co., N.Y., and Hunkapiller et al., *Nature*, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a polypeptide can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid,  $\alpha$ -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid,  $\gamma$ -Abu,  $\epsilon$ -Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine,  $\beta$ -alanine, fluoro-amino acids, designer amino acids such as  $\beta$ -methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

**[0358]** The invention encompasses albumin fusion proteins of the present invention which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH<sub>4</sub>; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

**[0359]** Additional post-translational modifications encompassed by the invention

include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The albumin fusion proteins may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

**[0360]** Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include iodine ( $^{121}\text{I}$ ,  $^{123}\text{I}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{111}\text{In}$ ,  $^{112}\text{In}$ ,  $^{113\text{m}}\text{In}$ ,  $^{115\text{m}}\text{In}$ ), technetium ( $^{99}\text{Tc}$ ,  $^{99\text{m}}\text{Tc}$ ), thallium ( $^{201}\text{Tl}$ ), gallium ( $^{68}\text{Ga}$ ,  $^{67}\text{Ga}$ ), palladium ( $^{103}\text{Pd}$ ), molybdenum ( $^{99}\text{Mo}$ ), xenon ( $^{133}\text{Xe}$ ), fluorine ( $^{18}\text{F}$ ),  $^{153}\text{Sm}$ ,  $^{177}\text{Lu}$ ,  $^{159}\text{Gd}$ ,  $^{149}\text{Pm}$ ,  $^{140}\text{La}$ ,  $^{175}\text{Yb}$ ,  $^{166}\text{Ho}$ ,  $^{90}\text{Y}$ ,  $^{47}\text{Sc}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$ ,  $^{142}\text{Pr}$ ,  $^{105}\text{Rh}$ , and  $^{97}\text{Ru}$ .

**[0361]** In specific embodiments, albumin fusion proteins of the present invention or fragments or variants thereof are attached to macrocyclic chelators that associate with radiometal ions, including but not limited to,  $^{177}\text{Lu}$ ,  $^{90}\text{Y}$ ,  $^{166}\text{Ho}$ , and  $^{153}\text{Sm}$ , to polypeptides. In a preferred embodiment, the radiometal ion associated with the macrocyclic chelators is  $^{111}\text{In}$ .

In another preferred embodiment, the radiometal ion associated with the macrocyclic chelator is  $^{90}\text{Y}$ . In specific embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA). In other specific embodiments, DOTA is attached to an antibody of the invention or fragment thereof via linker molecule. Examples of linker molecules useful for conjugating DOTA to a polypeptide are commonly known in the art - see, for example, DeNardo et al., Clin Cancer Res. 4(10):2483-90 (1998); Peterson et al., Bioconjug. Chem. 10(4):553-7 (1999); and Zimmerman et al, Nucl. Med. Biol. 26(8):943-50 (1999); which are hereby incorporated by reference in their entirety.

**[0362]** As mentioned, the albumin fusion proteins of the invention may be modified by either natural processes, such as post-translational processing, or by chemical modification techniques which are well known in the art. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given

polypeptide. Polypeptides of the invention may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, *PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES*, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); *POST-TRANSLATIONAL COVALENT MODIFICATION OF PROTEINS*, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., *Meth. Enzymol.* 182:626-646 (1990); Rattan et al., *Ann. N.Y. Acad. Sci.* 663:48-62 (1992)).

**[0363]** Albumin fusion proteins of the invention and antibodies that bind a Therapeutic protein or fragments or variants thereof can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., *Proc. Natl. Acad. Sci. USA* 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., *Cell* 37:767 (1984)) and the "flag" tag.

**[0364]** Further, an albumin fusion protein of the invention may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, <sup>213</sup>Bi. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy

anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis- dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

**[0365]** The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha-interferon,  $\beta$ -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF-alpha, TNF-beta, AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al.*, *Int. Immunol.*, 6:1567-1574 (1994)), VEGI (See, International Publication No. WO 99/23105), a thrombotic agent or an anti- angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors. Techniques for conjugating such therapeutic moiety to proteins (e.g., albumin fusion proteins) are well known in the art.

**[0366]** Albumin fusion proteins may also be attached to solid supports, which are particularly useful for immunoassays or purification of polypeptides that are bound by, that bind to, or associate with albumin fusion proteins of the invention. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

**[0367]** Albumin fusion proteins, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

**[0368]** In embodiments where the albumin fusion protein of the invention comprises only the VH domain of an antibody that binds a Therapeutic protein, it may be necessary and/or desirable to coexpress the fusion protein with the VL domain of the same antibody that binds a Therapeutic protein, such that the VH-albumin fusion protein and VL protein will associate (either covalently or non-covalently) post-translationally.

**[0369]** In embodiments where the albumin fusion protein of the invention comprises only the VL domain of an antibody that binds a Therapeutic protein, it may be necessary and/or desirable to coexpress the fusion protein with the VH domain of the same antibody that binds a Therapeutic protein, such that the VL-albumin fusion protein and VH protein will associate (either covalently or non-covalently) post-translationally.

**[0370]** Some Therapeutic antibodies are bispecific antibodies, meaning the antibody that binds a Therapeutic protein is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. In order to create an albumin fusion protein corresponding to that Therapeutic protein, it is possible to create an albumin fusion protein which has an scFv fragment fused to both the N- and C- terminus of the albumin protein moiety. More particularly, the scFv fused to the N-terminus of albumin would correspond to one of the heavy/light (VH/VL) pairs of the original antibody that binds a Therapeutic protein and the scFv fused to the C-terminus of albumin would correspond to the other heavy/light (VH/VL) pair of the original antibody that binds a Therapeutic protein.

**[0371]** Also provided by the invention are chemically modified derivatives of the albumin fusion proteins of the invention which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The albumin fusion proteins may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

**[0372]** The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic

profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a Therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 45,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

**[0373]** As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo *et al.*, *Appl. Biochem. Biotechnol.* 56:59-72 (1996); Vorobjev *et al.*, *Nucleosides Nucleotides* 18:2745-2750 (1999); and Caliceti *et al.*, *Bioconjug. Chem.* 10:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

**[0374]** The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, such as, for example, the method disclosed in EP 0 401 384 (coupling PEG to G-CSF), herein incorporated by reference; see also Malik *et al.*, *Exp. Hematol.* 20:1028-1035 (1992), reporting pegylation of GM-CSF using tresyl chloride. For example, polyethylene glycol may be covalently bound through amino acid residues via reactive group, such as a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

**[0375]** As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to proteins via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or



cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

**[0376]** One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

**[0377]** As indicated above, pegylation of the albumin fusion proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the albumin fusion protein either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado et al., *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992); Francis et al., *Intern. J. of Hematol.* 68:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

**[0378]** One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride ( $\text{ClSO}_2\text{CH}_2\text{CF}_3$ ). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoroethane sulphonyl group.

**[0379]** Polyethylene glycol can also be attached to proteins using a number of different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting

polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number of additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in International Publication No. WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated protein products produced using the reaction chemistries set out herein are included within the scope of the invention.

**[0380]** The number of polyethylene glycol moieties attached to each albumin fusion protein of the invention (i.e., the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado et al., *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992).

**[0381]** The polypeptides of the invention can be recovered and purified from chemical synthesis and recombinant cell cultures by standard methods which include, but are not limited to, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Well known techniques for refolding protein may be employed to regenerate active conformation when the polypeptide is denatured during isolation and/or purification.

**[0382]** The presence and quantity of albumin fusion proteins of the invention may be determined using ELISA, a well known immunoassay known in the art. In one ELISA protocol that would be useful for detecting/quantifying albumin fusion proteins of the invention, comprises the steps of coating an ELISA plate with an anti-human serum albumin antibody, blocking the plate to prevent non-specific binding, washing the ELISA plate, adding a solution containing the albumin fusion protein of the invention (at one or more different concentrations), adding a secondary anti-Therapeutic protein specific antibody

coupled to a detectable label (as described herein or otherwise known in the art), and detecting the presence of the secondary antibody. In an alternate version of this protocol, the ELISA plate might be coated with the anti-Therapeutic protein specific antibody and the labeled secondary reagent might be the anti-human albumin specific antibody.

### ***Uses of the Polynucleotides***

[0383] Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

[0384] The polynucleotides of the present invention are useful to produce the albumin fusion proteins of the invention. As described in more detail below, polynucleotides of the invention (encoding albumin fusion proteins) may be used in recombinant DNA methods useful in genetic engineering to make cells, cell lines, or tissues that express the albumin fusion protein encoded by the polynucleotides encoding albumin fusion proteins of the invention.

[0385] Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell. Additional non-limiting examples of gene therapy methods encompassed by the present invention are more thoroughly described elsewhere herein (see, e.g., the sections labeled "Gene Therapy", and Examples 63 and 64).

### ***Uses of the Polypeptides***

[0386] Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

[0387] Albumin fusion proteins of the invention are useful to provide immunological probes for differential identification of the tissue(s) (e.g., immunohistochemistry assays such as, for example, ABC immunoperoxidase (Hsu et al., J. Histochem. Cytochem. 29:577-580 (1981)) or cell type(s) (e.g., immunocytochemistry assays).

[0388] Albumin fusion proteins can be used to assay levels of polypeptides in a biological sample using classical immunohistological methods known to those of skill in the

art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell. Biol. 105:3087-3096 (1987)). Other methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine ( $^{131}\text{I}$ ,  $^{125}\text{I}$ ,  $^{123}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{115\text{m}}\text{In}$ ,  $^{113\text{m}}\text{In}$ ,  $^{112}\text{In}$ ,  $^{111}\text{In}$ ), and technetium ( $^{99}\text{Tc}$ ,  $^{99\text{m}}\text{Tc}$ ), thallium ( $^{201}\text{Ti}$ ), gallium ( $^{68}\text{Ga}$ ,  $^{67}\text{Ga}$ ), palladium ( $^{103}\text{Pd}$ ), molybdenum ( $^{99}\text{Mo}$ ), xenon ( $^{133}\text{Xe}$ ), fluorine ( $^{18}\text{F}$ ),  $^{153}\text{Sm}$ ,  $^{177}\text{Lu}$ ,  $^{159}\text{Gd}$ ,  $^{149}\text{Pm}$ ,  $^{140}\text{La}$ ,  $^{175}\text{Yb}$ ,  $^{166}\text{Ho}$ ,  $^{90}\text{Y}$ ,  $^{47}\text{Sc}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$ ,  $^{142}\text{Pr}$ ,  $^{105}\text{Rh}$ ,  $^{97}\text{Ru}$ ; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

**[0389]** Albumin fusion proteins of the invention can also be detected *in vivo* by imaging. Labels or markers for *in vivo* imaging of protein include those detectable by X-radiography, nuclear magnetic resonance (NMR) or electron spin relaxation (ESR). For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the albumin fusion protein by labeling of nutrients given to a cell line expressing the albumin fusion protein of the invention.

**[0390]** An albumin fusion protein which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example,  $^{131}\text{I}$ ,  $^{112}\text{In}$ ,  $^{99\text{m}}\text{Tc}$ ,  $^{131}\text{I}$ ,  $^{125}\text{I}$ ,  $^{123}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{115\text{m}}\text{In}$ ,  $^{113\text{m}}\text{In}$ ,  $^{112}\text{In}$ ,  $^{111}\text{In}$ ), and technetium ( $^{99}\text{Tc}$ ,  $^{99\text{m}}\text{Tc}$ ), thallium ( $^{201}\text{Ti}$ ), gallium ( $^{68}\text{Ga}$ ,  $^{67}\text{Ga}$ ), palladium ( $^{103}\text{Pd}$ ), molybdenum ( $^{99}\text{Mo}$ ), xenon ( $^{133}\text{Xe}$ ), fluorine ( $^{18}\text{F}$ ,  $^{153}\text{Sm}$ ,  $^{177}\text{Lu}$ ,  $^{159}\text{Gd}$ ,  $^{149}\text{Pm}$ ,  $^{140}\text{La}$ ,  $^{175}\text{Yb}$ ,  $^{166}\text{Ho}$ ,  $^{90}\text{Y}$ ,  $^{47}\text{Sc}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$ ,  $^{142}\text{Pr}$ ,  $^{105}\text{Rh}$ ,  $^{97}\text{Ru}$ ), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for immune system disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of  $^{99\text{m}}\text{Tc}$ . The labeled albumin fusion protein will then preferentially accumulate at locations in the body (e.g., organs, cells, extracellular spaces or matrices) where one or more receptors, ligands or substrates (corresponding to that of the Therapeutic protein used to make the albumin fusion protein of

the invention) are located. Alternatively, in the case where the albumin fusion protein comprises at least a fragment or variant of a Therapeutic antibody, the labeled albumin fusion protein will then preferentially accumulate at the locations in the body (e.g., organs, cells, extracellular spaces or matrices) where the polypeptides/epitopes corresponding to those bound by the Therapeutic antibody (used to make the albumin fusion protein of the invention) are located. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)). The protocols described therein could easily be modified by one of skill in the art for use with the albumin fusion proteins of the invention.

[0391] In one embodiment, the invention provides a method for the specific delivery of albumin fusion proteins of the invention to cells by administering albumin fusion proteins of the invention (e.g., polypeptides encoded by polynucleotides encoding albumin fusion proteins of the invention and/or antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a Therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

[0392] In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering albumin fusion proteins of the invention in association with toxins or cytotoxic prodrugs.

[0393] By "toxin" is meant one or more compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. "Toxin" also includes a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, <sup>213</sup>Bi, or other radioisotopes

such as, for example,  $^{103}\text{Pd}$ ,  $^{133}\text{Xe}$ ,  $^{131}\text{I}$ ,  $^{68}\text{Ge}$ ,  $^{57}\text{Co}$ ,  $^{65}\text{Zn}$ ,  $^{85}\text{Sr}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ ,  $^{90}\text{Y}$ ,  $^{153}\text{Sm}$ ,  $^{153}\text{Gd}$ ,  $^{169}\text{Yb}$ ,  $^{51}\text{Cr}$ ,  $^{54}\text{Mn}$ ,  $^{75}\text{Se}$ ,  $^{113}\text{Sn}$ ,  $^{90}\text{Yttrium}$ ,  $^{117}\text{Tin}$ ,  $^{186}\text{Rhenium}$ ,  $^{166}\text{Holmium}$ , and  $^{188}\text{Rhenium}$ ; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin. In a specific embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention or antibodies of the invention in association with the radioisotope  $^{90}\text{Y}$ . In another specific embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention or antibodies of the invention in association with the radioisotope  $^{111}\text{In}$ . In a further specific embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention or antibodies of the invention in association with the radioisotope  $^{131}\text{I}$ .

[0394] Techniques known in the art may be applied to label polypeptides of the invention. Such techniques include, but are not limited to, the use of bifunctional conjugating agents (see e.g., U.S. Patent Nos. 5,756,065; 5,714,631; 5,696,239; 5,652,361; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274,119; 4,994,560; and 5,808,003; the contents of each of which are hereby incorporated by reference in its entirety).

[0395] The albumin fusion proteins of the present invention are useful for diagnosis, treatment, prevention and/or prognosis of various disorders in mammals, preferably humans. Such disorders include, but are not limited to, those described herein under the section heading "Biological Activities," below.

[0396] Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression level of a certain polypeptide in cells or body fluid of an individual using an albumin fusion protein of the invention; and (b) comparing the assayed polypeptide expression level with a standard polypeptide expression level, whereby an increase or decrease in the assayed polypeptide expression level compared to the standard expression level is indicative of a disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

**[0397]** Moreover, albumin fusion proteins of the present invention can be used to treat or prevent diseases or conditions such as, for example, neural disorders, immune system disorders, muscular disorders, reproductive disorders, gastrointestinal disorders, pulmonary disorders, cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B, SOD, catalase, DNA repair proteins), to inhibit the activity of a polypeptide (e.g., an oncogene or tumor suppressor), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth inhibition, enhancement of the immune response to proliferative cells or tissues).

**[0398]** In particular, albumin fusion proteins comprising of at least a fragment or variant of a Therapeutic antibody can also be used to treat disease (as described *supra*, and elsewhere herein). For example, administration of an albumin fusion protein comprising of at least a fragment or variant of a Therapeutic antibody can bind, and/or neutralize the polypeptide to which the Therapeutic antibody used to make the albumin fusion protein specifically binds, and/or reduce overproduction of the polypeptide to which the Therapeutic antibody used to make the albumin fusion protein specifically binds. Similarly, administration of an albumin fusion protein comprising of at least a fragment or variant of a Therapeutic antibody can activate the polypeptide to which the Therapeutic antibody used to make the albumin fusion protein specifically binds, by binding to the polypeptide bound to a membrane (receptor).

**[0399]** At the very least, the albumin fusion proteins of the invention of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Albumin fusion proteins of the invention can also be used to raise antibodies, which in turn may be used to measure protein expression of the Therapeutic protein, albumin protein, and/or the albumin fusion protein of the invention from a recombinant cell, as a way of assessing transformation of the host cell, or in a biological sample. Moreover, the albumin fusion proteins of the present invention can be used to test the biological activities described herein.

### ***Diagnostic Assays***

**[0400]** The compounds of the present invention are useful for diagnosis, treatment, prevention and/or prognosis of various disorders in mammals, preferably humans. Such disorders include, but are not limited to, those described for each Therapeutic protein in the corresponding row of Table 1 and herein under the section headings “Immune Activity,” “Blood Related Disorders,” “Hyperproliferative Disorders,” “Renal Disorders,” “Cardiovascular Disorders,” “Respiratory Disorders,” “Anti-Angiogenesis Activity,” “Diseases at the Cellular Level,” “Wound Healing and Epithelial Cell Proliferation,” “Neural Activity and Neurological Diseases,” “Endocrine Disorders,” “Reproductive System Disorders,” “Infectious Disease,” “Regeneration,” and/or “Gastrointestinal Disorders,” *infra*.

**[0401]** For a number of disorders, substantially altered (increased or decreased) levels of gene expression can be detected in tissues, cells or bodily fluids (e.g., sera, plasma, urine, semen, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a “standard” gene expression level, that is, the expression level in tissues or bodily fluids from an individual not having the disorder. Thus, the invention provides a diagnostic method useful during diagnosis of a disorder, which involves measuring the expression level of the gene encoding a polypeptide in tissues, cells or body fluid from an individual and comparing the measured gene expression level with a standard gene expression level, whereby an increase or decrease in the gene expression level(s) compared to the standard is indicative of a disorder. These diagnostic assays may be performed *in vivo* or *in vitro*, such as, for example, on blood samples, biopsy tissue or autopsy tissue.

**[0402]** The present invention is also useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed gene expression will experience a worse clinical outcome

**[0403]** By “assaying the expression level of the gene encoding a polypeptide” is intended qualitatively or quantitatively measuring or estimating the level of a particular polypeptide (e.g. a polypeptide corresponding to a Therapeutic protein disclosed in Table 1) or the level of the mRNA encoding the polypeptide of the invention in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the polypeptide level or mRNA level in a second biological sample). Preferably, the polypeptide expression level or mRNA level in the first biological sample is measured or estimated and compared to a standard polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population



of individuals not having the disorder. As will be appreciated in the art, once a standard polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

**[0404]** By "biological sample" is intended any biological sample obtained from an individual, cell line, tissue culture, or other source containing polypeptides of the invention (including portions thereof) or mRNA. As indicated, biological samples include body fluids (such as sera, plasma, urine, synovial fluid and spinal fluid) and tissue sources found to express the full length or fragments thereof of a polypeptide or mRNA. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

**[0405]** Total cellular RNA can be isolated from a biological sample using any suitable technique such as the single-step guanidinium-thiocyanate-phenol-chloroform method described in Chomczynski and Sacchi, *Anal. Biochem.* 162:156-159 (1987). Levels of mRNA encoding the polypeptides of the invention are then assayed using any appropriate method. These include Northern blot analysis, S1 nuclease mapping, the polymerase chain reaction (PCR), reverse transcription in combination with the polymerase chain reaction (RT-PCR), and reverse transcription in combination with the ligase chain reaction (RT-LCR).

**[0406]** The present invention also relates to diagnostic assays such as quantitative and diagnostic assays for detecting levels of polypeptides that bind to, are bound by, or associate with albumin fusion proteins of the invention, in a biological sample (e.g., cells and tissues), including determination of normal and abnormal levels of polypeptides. Thus, for instance, a diagnostic assay in accordance with the invention for detecting abnormal expression of polypeptides that bind to, are bound by, or associate with albumin fusion proteins compared to normal control tissue samples may be used to detect the presence of tumors. Assay techniques that can be used to determine levels of a polypeptide that bind to, are bound by, or associate with albumin fusion proteins of the present invention in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays. Assaying polypeptide levels in a biological sample can occur using any art-known method.

**[0407]** Assaying polypeptide levels in a biological sample can occur using a variety of techniques. For example, polypeptide expression in tissues can be studied with classical immunohistological methods (Jalkanen et al., *J. Cell. Biol.* 101:976-985 (1985); Jalkanen, M., et al., *J. Cell . Biol.* 105:3087-3096 (1987)). Other methods useful for detecting

polypeptide gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine ( $^{125}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{112}\text{In}$ ), and technetium ( $^{99\text{m}}\text{Tc}$ ), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

**[0408]** The tissue or cell type to be analyzed will generally include those which are known, or suspected, to express the gene of interest (such as, for example, cancer). The protein isolation methods employed herein may, for example, be such as those described in Harlow and Lane (Harlow, E. and Lane, D., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York), which is incorporated herein by reference in its entirety. The isolated cells can be derived from cell culture or from a patient. The analysis of cells taken from culture may be a necessary step in the assessment of cells that could be used as part of a cell-based gene therapy technique or, alternatively, to test the effect of compounds on the expression of the gene.

**[0409]** For example, albumin fusion proteins may be used to quantitatively or qualitatively detect the presence of polypeptides that bind to, are bound by, or associate with albumin fusion proteins of the present invention. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled albumin fusion protein coupled with light microscopic, flow cytometric, or fluorimetric detection.

**[0410]** In a preferred embodiment, albumin fusion proteins comprising at least a fragment or variant of an antibody that specifically binds at least a Therapeutic protein disclosed herein (e.g., the Therapeutic proteins disclosed in Table 1) or otherwise known in the art may be used to quantitatively or qualitatively detect the presence of gene products or conserved variants or peptide fragments thereof. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled antibody coupled with light microscopic, flow cytometric, or fluorimetric detection.

**[0411]** The albumin fusion proteins of the present invention may, additionally, be employed histologically, as in immunofluorescence, immunoelectron microscopy or non-immunological assays, for in situ detection of polypeptides that bind to, are bound by, or associate with an albumin fusion protein of the present invention. In situ detection may be accomplished by removing a histological specimen from a patient, and applying thereto a labeled antibody or polypeptide of the present invention. The albumin fusion proteins are preferably applied by overlaying the labeled albumin fusion proteins onto a biological

sample. Through the use of such a procedure, it is possible to determine not only the presence of the polypeptides that bind to, are bound by, or associate with albumin fusion proteins, but also its distribution in the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such in situ detection.

**[0412]** Immunoassays and non-immunoassays that detect polypeptides that bind to, are bound by, or associate with albumin fusion proteins will typically comprise incubating a sample, such as a biological fluid, a tissue extract, freshly harvested cells, or lysates of cells which have been incubated in cell culture, in the presence of a detectably labeled antibody capable of binding gene products or conserved variants or peptide fragments thereof, and detecting the bound antibody by any of a number of techniques well-known in the art.

**[0413]** The biological sample may be brought in contact with and immobilized onto a solid phase support or carrier such as nitrocellulose, or other solid support which is capable of immobilizing cells, cell particles or soluble proteins. The support may then be washed with suitable buffers followed by treatment with the detectably labeled albumin fusion protein of the invention. The solid phase support may then be washed with the buffer a second time to remove unbound antibody or polypeptide. Optionally the antibody is subsequently labeled. The amount of bound label on solid support may then be detected by conventional means.

**[0414]** By "solid phase support or carrier" is intended any support capable of binding a polypeptide (e.g., an albumin fusion protein, or polypeptide that binds, is bound by, or associates with an albumin fusion protein of the invention.) Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to a polypeptide. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

**[0415]** The binding activity of a given lot of albumin fusion protein may be determined according to well known methods. Those skilled in the art will be able to

determine operative and optimal assay conditions for each determination by employing routine experimentation.

[0416] In addition to assaying polypeptide levels in a biological sample obtained from an individual, polypeptide can also be detected *in vivo* by imaging. For example, in one embodiment of the invention, albumin fusion proteins of the invention are used to image diseased or neoplastic cells.

[0417] Labels or markers for *in vivo* imaging of albumin fusion proteins of the invention include those detectable by X-radiography, NMR, MRI, CAT-scans or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the albumin fusion protein by labeling of nutrients of a cell line (or bacterial or yeast strain) engineered.

[0418] Additionally, albumin fusion proteins of the invention whose presence can be detected, can be administered. For example, albumin fusion proteins of the invention labeled with a radio-opaque or other appropriate compound can be administered and visualized *in vivo*, as discussed, above for labeled antibodies. Further, such polypeptides can be utilized for *in vitro* diagnostic procedures.

[0419] A polypeptide-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example,  $^{131}\text{I}$ ,  $^{112}\text{In}$ ,  $^{99\text{m}}\text{Tc}$ ), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for a disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of  $^{99\text{m}}\text{Tc}$ . The labeled albumin fusion protein will then preferentially accumulate at the locations in the body which contain a polypeptide or other substance that binds to, is bound by or associates with an albumin fusion protein of the present invention. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

[0420] One of the ways in which an albumin fusion protein of the present invention

can be detectably labeled is by linking the same to a reporter enzyme and using the linked product in an enzyme immunoassay (EIA) (Voller, A., "The Enzyme Linked Immunosorbent Assay (ELISA)", 1978, Diagnostic Horizons 2:1-7, Microbiological Associates Quarterly Publication, Walkersville, MD); Voller et al., *J. Clin. Pathol.* 31:507-520 (1978); Butler, J.E., *Meth. Enzymol.* 73:482-523 (1981); Maggio, E. (ed.), 1980, Enzyme Immunoassay, CRC Press, Boca Raton, FL.; Ishikawa, E. et al., (eds.), 1981, Enzyme Immunoassay, Kigaku Shoin, Tokyo). The reporter enzyme which is bound to the antibody will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorimetric or by visual means. Reporter enzymes which can be used to detectably label the antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. Additionally, the detection can be accomplished by colorimetric methods which employ a chromogenic substrate for the reporter enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

**[0421]** Albumin fusion proteins may also be radiolabelled and used in any of a variety of other immunoassays. For example, by radioactively labeling the albumin fusion proteins, it is possible to use the albumin fusion proteins in a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope can be detected by means including, but not limited to, a gamma counter, a scintillation counter, or autoradiography.

**[0422]** Additionally, chelator molecules, are known in the art and can be used to label the Albumin fusion proteins. Chelator molecules may be attached to Albumin fusion proteins of the invention to facilitate labeling said protein with metal ions including radionuclides or fluorescent labels. For example, see Subramanian, R. and Meares, C.F., "Bifunctional Chelating Agents for Radiometal-labeled monoclonal Antibodies," in *Cancer Imaging with Radiolabeled Antibodies* (D. M. Goldenberg, Ed.) Kluwer Academic Publications, Boston; Saji, H., "Targeted delivery of radiolabeled imaging and therapeutic agents: bifunctional radiopharmaceuticals." *Crit. Rev. Ther. Drug Carrier Syst.* 16:209-244 (1999); Srivastava

S.C. and Mease R.C., "Progress in research on ligands, nuclides and techniques for labeling monoclonal antibodies." *Int. J. Rad. Appl. Instrum. B* 18:589-603 (1991); and Liu, S. and Edwards, D.S., "Bifunctional chelators for therapeutic lanthanide radiopharmaceuticals." *Bioconjug. Chem.* 12:7-34 (2001). Any chelator which can be covalently bound to said Albumin fusion proteins may be used according to the present invention. The chelator may further comprise a linker moiety that connects the chelating moiety to the Albumin fusion protein.

**[0423]** In one embodiment, the Albumin fusion protein of the invention are attached to an acyclic chelator such as diethylene triamine-N,N,N',N'',N'''-pentaacetic acid (DPTA), analogues of DPTA, and derivatives of DPTA. As non-limiting examples, the chelator may be 2-(p-isothiocyanatobenzyl)-6- methyl-diethylenetriaminepentaacetic acid (1B4M-DPTA, also known as MX-DTPA), 2-methyl-6-(rho-nitrobenzyl)-1,4,7- triazaheptane-N,N,N',N'',N'''-pentaacetic acid (nitro-1B4M-DTPA or nitro-MX-DTPA); 2-(p-isothiocyanatobenzyl)-cyclohexyldiethylenetriaminepentaacetic acid (CHX-DTPA), or N-[2-amino-3-(rho-nitrophenyl)propyl]-trans-cyclohexane-1,2-diamine-N,N',N''-pentaacetic acid (nitro-CHX-A-DTPA).

**[0424]** In another embodiment, the Albumin fusion protein of the invention are attached to an acyclic terpyridine chelator such as 6,6''-bis[[N,N,N'',N'''-tetra(carboxymethyl)amino]methyl]-4'-(3-amino-4-methoxyphenyl)-2,2':6',2''- terpyridine (TMT-amine).

**[0425]** In specific embodiments, the macrocyclic chelator which is attached to the the Albumin fusion protein of the invention is 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA). In other specific embodiments, the DOTA is attached to the the Albumin fusion protein of the invention via a linker molecule. Examples of linker molecules useful for conjugating DOTA to a polypeptide are commonly known in the art - see, for example, DeNardo *et al.*, *Clin. Cancer Res.* 4(10):2483-90, 1998; Peterson *et al.*, *Bioconjug. Chem.* 10(4):553-7, 1999; and Zimmerman *et al.*, *Nucl. Med. Biol.* 26(8):943-50, 1999 which are hereby incorporated by reference in their entirety. In addition, U.S. Patents 5,652,361 and 5,756,065, which disclose chelating agents that may be conjugated to antibodies, and methods for making and using them, are hereby incorporated by reference in their entireties. Though U.S. Patents 5,652,361 and 5,756,065 focus on conjugating chelating agents to antibodies, one skilled in the art could readily adapt the method disclosed therein in order to conjugate chelating agents to other polypeptides.

[0426] Bifunctional chelators based on macrocyclic ligands in which conjugation is via an activated arm, or functional group, attached to the carbon backbone of the ligand can be employed as described by M. Moi *et al.*, *J. Amer. Chem. Soc.* 49:2639 (1989) (2-*p*-nitrobenzyl-1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid); S. V. Deshpande *et al.*, *J. Nucl. Med.* 31:473 (1990); G. Ruser *et al.*, *Bioconj. Chem.* 1:345 (1990); C. J. Broan *et al.*, *J. C. S. Chem. Comm.* 23:1739 (1990); and C. J. Anderson *et al.*, *J. Nucl. Med.* 36:850 (1995).

[0427] In one embodiment, a macrocyclic chelator, such as polyazamacrocyclic chelators, optionally containing one or more carboxy, amino, hydroxamate, phosphonate, or phosphate groups, are attached to the Albumin fusion protein of the invention. In another embodiment, the chelator is a chelator selected from the group consisting of DOTA, analogues of DOTA, and derivatives of DOTA.

[0428] In one embodiment, suitable chelator molecules that may be attached to the the Albumin fusion protein of the invention include DOXA (1-oxa-4,7,10-triazacyclododecanetriacetic acid), NOTA (1,4,7-triazacyclononanetriacetic acid), TETA (1,4,8,11-tetraazacyclotetradecanetetraacetic acid), and THT (4'-(3-amino-4-methoxyphenyl)-6,6''-bis(*N',N'*-dicarboxymethyl-*N*-methylhydrazino)-2,2':6',2''-terpyridine), and analogs and derivatives thereof. See, *e.g.*, Ohmono *et al.*, *J. Med. Chem.* 35: 157-162 (1992); Kung *et al.*, *J. Nucl. Med.* 25: 326-332 (1984); Jurisson *et al.*, *Chem. Rev.* 93:1137-1156 (1993); and U.S. Patent No. 5,367,080. Other suitable chelators include chelating agents disclosed in U.S. Patent Nos. 4,647,447; 4,687,659; 4,885,363; EP-A-71564; WO89/00557; and EP-A-232751.

[0429] In another embodiment, suitable macrocyclic carboxylic acid chelators which can be used in the present invention include 1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid (DOTA); 1,4,8,12-tetraazacyclopentadecane-*N,N',N'',N'''*-tetraacetic acid (15N4); 1,4,7-triazacyclononane-*N,N',N''*-triacetic acid (9N3); 1,5,9-triazacyclododecane-*N,N',N''*-triacetic acid (12N3); and 6-bromoacetamido-benzyl-1,4,8,11-tetraazacyclotetradecane-*N,N',N'',N'''*-tetraacetic acid (BAT).

[0430] A preferred chelator that can be attached to the Albumin Fusion protein of the invention is  $\alpha$ -(5-isothiocyanato-2-methoxyphenyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, which is also known as MeO-DOTA-NCS. A salt or ester of  $\alpha$ -(5-isothiocyanato-2-methoxyphenyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid may also be used.

[0431] Albumin fusion proteins of the invention to which chelators such as those described are covalently attached may be labeled (via the coordination site of the chelator) with radionuclides that are suitable for therapeutic, diagnostic, or both therapeutic and diagnostic purposes. Examples of appropriate metals include Ag, At, Au, Bi, Cu, Ga, Ho, In, Lu, Pb, Pd, Pm, Pr, Rb, Re, Rh, Sc, Sr, Tc, Tl, Y, and Yb. Examples of the radionuclide used for diagnostic purposes are Fe, Gd,  $^{111}\text{In}$ ,  $^{67}\text{Ga}$ , or  $^{68}\text{Ga}$ . In another embodiment, the radionuclide used for diagnostic purposes is  $^{111}\text{In}$ , or  $^{67}\text{Ga}$ . Examples of the radionuclide used for therapeutic purposes are  $^{166}\text{Ho}$ ,  $^{165}\text{Dy}$ ,  $^{90}\text{Y}$ ,  $^{115\text{m}}\text{In}$ ,  $^{52}\text{Fe}$ , or  $^{72}\text{Ga}$ . In one embodiment, the radionuclide used for diagnostic purposes is  $^{166}\text{Ho}$  or  $^{90}\text{Y}$ . Examples of the radionuclides used for both therapeutic and diagnostic purposes include  $^{153}\text{Sm}$ ,  $^{177}\text{Lu}$ ,  $^{159}\text{Gd}$ ,  $^{175}\text{Yb}$ , or  $^{47}\text{Sc}$ . In one embodiment, the radionuclide is  $^{153}\text{Sm}$ ,  $^{177}\text{Lu}$ ,  $^{175}\text{Yb}$ , or  $^{159}\text{Gd}$ .

[0432] Preferred metal radionuclides include  $^{90}\text{Y}$ ,  $^{99\text{m}}\text{Tc}$ ,  $^{111}\text{In}$ ,  $^{47}\text{Sc}$ ,  $^{67}\text{Ga}$ ,  $^{51}\text{Cr}$ ,  $^{177\text{m}}\text{Sn}$ ,  $^{67}\text{Cu}$ ,  $^{167}\text{Tm}$ ,  $^{97}\text{Ru}$ ,  $^{188}\text{Re}$ ,  $^{177}\text{Lu}$ ,  $^{199}\text{Au}$ ,  $^{47}\text{Sc}$ ,  $^{67}\text{Ga}$ ,  $^{51}\text{Cr}$ ,  $^{177\text{m}}\text{Sn}$ ,  $^{67}\text{Cu}$ ,  $^{167}\text{Tm}$ ,  $^{95}\text{Ru}$ ,  $^{188}\text{Re}$ ,  $^{177}\text{Lu}$ ,  $^{199}\text{Au}$ ,  $^{203}\text{Pb}$  and  $^{141}\text{Ce}$ .

[0433] In a particular embodiment, Albumin fusion proteins of the invention to which chelators are covalently attached may be labeled with a metal ion selected from the group consisting of  $^{90}\text{Y}$ ,  $^{111}\text{In}$ ,  $^{177}\text{Lu}$ ,  $^{166}\text{Ho}$ ,  $^{215}\text{Bi}$ , and  $^{225}\text{Ac}$ .

[0434] Moreover,  $\gamma$ -emitting radionuclides, such as  $^{99\text{m}}\text{Tc}$ ,  $^{111}\text{In}$ ,  $^{67}\text{Ga}$ , and  $^{169}\text{Yb}$  have been approved or under investigation for diagnostic imaging, while  $\beta$ -emitters, such as  $^{67}\text{Cu}$ ,  $^{111}\text{Ag}$ ,  $^{186}\text{Re}$ , and  $^{90}\text{Y}$  are useful for the applications in tumor therapy. Also other useful radionuclides include  $\gamma$ -emitters, such as  $^{99\text{m}}\text{Tc}$ ,  $^{111}\text{In}$ ,  $^{67}\text{Ga}$ , and  $^{169}\text{Yb}$ , and  $\beta$ -emitters, such as  $^{67}\text{Cu}$ ,  $^{111}\text{Ag}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$  and  $^{90}\text{Y}$ , as well as other radionuclides of interest such as  $^{211}\text{At}$ ,  $^{212}\text{Bi}$ ,  $^{177}\text{Lu}$ ,  $^{86}\text{Rb}$ ,  $^{105}\text{Rh}$ ,  $^{153}\text{Sm}$ ,  $^{198}\text{Au}$ ,  $^{149}\text{Pm}$ ,  $^{85}\text{Sr}$ ,  $^{142}\text{Pr}$ ,  $^{214}\text{Pb}$ ,  $^{109}\text{Pd}$ ,  $^{166}\text{Ho}$ ,  $^{208}\text{Tl}$ , and  $^{44}\text{Sc}$ .

Albumin fusion proteins of the invention to which chelators are covalently attached may be labeled with the radionuclides described above.

[0435] In another embodiment, Albumin fusion proteins of the invention to which chelators are covalently attached may be labeled with paramagnetic metal ions including ions of transition and lanthanide metal, such as metals having atomic numbers of 21-29, 42, 43, 44, or 57-71, in particular ions of Cr, V, Mn, Fe, Co, Ni, Cu, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu. The paramagnetic metals used in compositions for magnetic resonance imaging include the elements having atomic numbers of 22 to 29, 42, 44 and 58-70.



[0436] In another embodiment, Albumin fusion proteins of the invention to which chelators are covalently attached may be labeled with fluorescent metal ions including lanthanides, in particular La, Ce, Pr, Nd, Pm, Sm, Eu (e.g.,  $^{152}\text{Eu}$ ), Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu.

[0437] In another embodiment, Albumin fusion proteins of the invention to which chelators are covalently attached may be labeled with heavy metal-containing reporters may include atoms of Mo, Bi, Si, and W.

[0438] It is also possible to label the albumin fusion proteins with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, ophthaldehyde and fluorescamine.

[0439] The albumin fusion protein can also be detectably labeled using fluorescence emitting metals such as  $^{152}\text{Eu}$ , or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

[0440] The albumin fusion proteins can also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged albumin fusion protein is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

[0441] Likewise, a bioluminescent compound may be used to label albumin fusion proteins of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

### ***Transgenic Organisms***

[0442] Transgenic organisms that express the albumin fusion proteins of the invention are also included in the invention. Transgenic organisms are genetically modified organisms into which recombinant, exogenous or cloned genetic material has been

transferred. Such genetic material is often referred to as a transgene. The nucleic acid sequence of the transgene may include one or more transcriptional regulatory sequences and other nucleic acid sequences such as introns, that may be necessary for optimal expression and secretion of the encoded protein. The transgene may be designed to direct the expression of the encoded protein in a manner that facilitates its recovery from the organism or from a product produced by the organism, *e.g.* from the milk, blood, urine, eggs, hair or seeds of the organism. The transgene may consist of nucleic acid sequences derived from the genome of the same species or of a different species than the species of the target animal. The transgene may be integrated either at a locus of a genome where that particular nucleic acid sequence is not otherwise normally found or at the normal locus for the transgene.

**[0443]** The term “germ cell line transgenic organism” refers to a transgenic organism in which the genetic alteration or genetic information was introduced into a germ line cell, thereby conferring the ability of the transgenic organism to transfer the genetic information to offspring. If such offspring in fact possess some or all of that alteration or genetic information, then they too are transgenic organisms. The alteration or genetic information may be foreign to the species of organism to which the recipient belongs, foreign only to the particular individual recipient, or may be genetic information already possessed by the recipient. In the last case, the altered or introduced gene may be expressed differently than the native gene.

**[0444]** A transgenic organism may be a transgenic animal or a transgenic plant. Transgenic animals can be produced by a variety of different methods including transfection, electroporation, microinjection, gene targeting in embryonic stem cells and recombinant viral and retroviral infection (*see, e.g.*, U.S. Patent No. 4,736,866; U.S. Patent No. 5,602,307; Mullins *et al.* (1993) *Hypertension* 22(4):630-633; Brenin *et al.* (1997) *Surg. Oncol.* 6(2)99-110; Tuan (ed.), *Recombinant Gene Expression Protocols*, Methods in Molecular Biology No. 62, Humana Press (1997)). The method of introduction of nucleic acid fragments into recombination competent mammalian cells can be by any method which favors co-transformation of multiple nucleic acid molecules. Detailed procedures for producing transgenic animals are readily available to one skilled in the art, including the disclosures in U.S. Patent No. 5,489,743 and U.S. Patent No. 5,602,307.

**[0445]** A number of recombinant or transgenic mice have been produced, including those which express an activated oncogene sequence (U.S. Patent No. 4,736,866); express simian SV40 T-antigen (U.S. Patent No. 5,728,915); lack the expression of interferon

regulatory factor 1 (IRF-1) (U.S. Patent No. 5,731,490); exhibit dopaminergic dysfunction (U.S. Patent No. 5,723,719); express at least one human gene which participates in blood pressure control (U.S. Patent No. 5,731,489); display greater similarity to the conditions existing in naturally occurring Alzheimer's disease (U.S. Patent No. 5,720,936); have a reduced capacity to mediate cellular adhesion (U.S. Patent No. 5,602,307); possess a bovine growth hormone gene (Clutter *et al.* (1996) *Genetics* 143(4):1753-1760); or, are capable of generating a fully human antibody response (McCarthy (1997) *The Lancet* 349(9049):405).

[0446] While mice and rats remain the animals of choice for most transgenic experimentation, in some instances it is preferable or even necessary to use alternative animal species. Transgenic procedures have been successfully utilized in a variety of non-murine animals, including sheep, goats, pigs, dogs, cats, monkeys, chimpanzees, hamsters, rabbits, cows and guinea pigs (*see, e.g.,* Kim *et al.* (1997) *Mol. Reprod. Dev.* 46(4):515-526; Houdebine (1995) *Reprod. Nutr. Dev.* 35(6):609-617; Petters (1994) *Reprod. Fertil. Dev.* 6(5):643-645; Schnieke *et al.* (1997) *Science* 278(5346):2130-2133; and Amoah (1997) *J. Animal Science* 75(2):578-585).

[0447] To direct the secretion of the transgene-encoded protein of the invention into the milk of transgenic mammals, it may be put under the control of a promoter that is preferentially activated in mammary epithelial cells. Promoters that control the genes encoding milk proteins are preferred, for example the promoter for casein, beta lactoglobulin, whey acid protein, or lactalbumin (*see, e.g.,* DiTullio (1992) *BioTechnology* 10:74-77; Clark *et al.* (1989) *BioTechnology* 7:487-492; Gorton *et al.* (1987) *BioTechnology* 5:1183-1187; and Soulier *et al.* (1992) *FEBS Letts.* 297:13). The transgenic mammals of choice would produce large volumes of milk and have long lactating periods, for example goats, cows, camels or sheep.

[0448] An albumin fusion protein of the invention can also be expressed in a transgenic plant, *e.g.* a plant in which the DNA transgene is inserted into the nuclear or plastidic genome. Plant transformation procedures used to introduce foreign nucleic acids into plant cells or protoplasts are known in the art. *See, in general, Methods in Enzymology* Vol. 153 ("Recombinant DNA Part D") 1987, Wu and Grossman Eds., Academic Press and European Patent Application EP 693554. Methods for generation of genetically engineered plants are further described in US Patent No. 5,283,184, US Patent No. 5,482,852, and European Patent Application EP 693 554, all of which are hereby incorporated by reference.

### ***Pharmaceutical or Therapeutic Compositions***

[0449] The albumin fusion proteins of the invention or formulations thereof may be administered by any conventional method including parenteral (*e.g.* subcutaneous or intramuscular) injection or intravenous infusion. The treatment may consist of a single dose or a plurality of doses over a period of time.

[0450] While it is possible for an albumin fusion protein of the invention to be administered alone, it is preferable to present it as a pharmaceutical formulation, together with one or more acceptable carriers. The carrier(s) must be "acceptable" in the sense of being compatible with the albumin fusion protein and not deleterious to the recipients thereof. Typically, the carriers will be water or saline which will be sterile and pyrogen free. Albumin fusion proteins of the invention are particularly well suited to formulation in aqueous carriers such as sterile pyrogen free water, saline or other isotonic solutions because of their extended shelf-life in solution. For instance, pharmaceutical compositions of the invention may be formulated well in advance in aqueous form, for instance, weeks or months or longer time periods before being dispensed.

[0451] For example, formulations containing the albumin fusion protein may be prepared taking into account the extended shelf-life of the albumin fusion protein in aqueous formulations. As discussed above, the shelf-life of many of these Therapeutic proteins are markedly increased or prolonged after fusion to HA.

[0452] In instances where aerosol administration is appropriate, the albumin fusion proteins of the invention can be formulated as aerosols using standard procedures. The term "aerosol" includes any gas-borne suspended phase of an albumin fusion protein of the instant invention which is capable of being inhaled into the bronchioles or nasal passages. Specifically, aerosol includes a gas-borne suspension of droplets of an albumin fusion protein of the instant invention, as may be produced in a metered dose inhaler or nebulizer, or in a mist sprayer. Aerosol also includes a dry powder composition of a compound of the instant invention suspended in air or other carrier gas, which may be delivered by insufflation from an inhaler device, for example. See Ganderton & Jones, *Drug Delivery to the Respiratory Tract*, Ellis Horwood (19 87); Gonda (1990) *Critical Reviews in Therapeutic Drug Carrier Systems* 6:273-313; and Raeburn *et al.*, (1992) *Pharmacol. Toxicol. Methods* 27:143-159.

[0453] The formulations of the invention are also typically non-immunogenic, in part, because of the use of the components of the albumin fusion protein being derived from the proper species. For instance, for human use, both the Therapeutic protein and albumin

portions of the albumin fusion protein will typically be human. In some cases, wherein either component is non human-derived, that component may be humanized by substitution of key amino acids so that specific epitopes appear to the human immune system to be human in nature rather than foreign.

**[0454]** The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the albumin fusion protein with the carrier that constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

**[0455]** Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation appropriate for the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampules, vials or syringes, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders. Dosage formulations may contain the Therapeutic protein portion at a lower molar concentration or lower dosage compared to the non-fused standard formulation for the Therapeutic protein given the extended serum half-life exhibited by many of the albumin fusion proteins of the invention.

**[0456]** As an example, when an albumin fusion protein of the invention comprises one of the proteins listed in the "Therapeutic Protein:X" column of Table 1 as one or more of the Therapeutic protein regions, the dosage form can be calculated on the basis of the potency of the albumin fusion protein relative to the potency of hGH, while taking into account the prolonged serum half-life and shelf-life of the albumin fusion proteins compared to that of native hGH. Growth hormone is typically administered at 0.3 to 30.0 IU/kg/week, for example 0.9 to 12.0 IU/kg/week, given in three or seven divided doses for a year or more. In an albumin fusion protein consisting of full length HA fused to full length GH, an equivalent dose in terms of units would represent a greater weight of agent but the dosage frequency can be reduced, for example to twice a week, once a week or less.

**[0457]** Formulations or compositions of the invention may be packaged together with,

or included in a kit with, instructions or a package insert referring to the extended shelf-life of the albumin fusion protein component. For instance, such instructions or package inserts may address recommended storage conditions, such as time, temperature and light, taking into account the extended or prolonged shelf-life of the albumin fusion proteins of the invention. Such instructions or package inserts may also address the particular advantages of the albumin fusion proteins of the inventions, such as the ease of storage for formulations that may require use in the field, outside of controlled hospital, clinic or office conditions. As described above, formulations of the invention may be in aqueous form and may be stored under less than ideal circumstances without significant loss of therapeutic activity.

**[0458]** Albumin fusion proteins of the invention can also be included in nutraceuticals. For instance, certain albumin fusion proteins of the invention may be administered in natural products, including milk or milk product obtained from a transgenic mammal which expresses albumin fusion protein. Such compositions can also include plant or plant products obtained from a transgenic plant which expresses the albumin fusion protein. The albumin fusion protein can also be provided in powder or tablet form, with or without other known additives, carriers, fillers and diluents. Nutraceuticals are described in Scott Hegenhart, *Food Product Design*, Dec. 1993.

**[0459]** The invention also provides methods of treatment and/or prevention of diseases or disorders (such as, for example, any one or more of the diseases or disorders disclosed herein) by administration to a subject of an effective amount of an albumin fusion protein of the invention or a polynucleotide encoding an albumin fusion protein of the invention ("albumin fusion polynucleotide") in a pharmaceutically acceptable carrier.

**[0460]** The albumin fusion protein and/or polynucleotide will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the albumin fusion protein and/or polynucleotide alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

**[0461]** As a general proposition, the total pharmaceutically effective amount of the albumin fusion protein administered parenterally per dose will be in the range of about 1 µg/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given

continuously, the albumin fusion protein is typically administered at a dose rate of about 1 ug/kg/hour to about 50 ug/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

**[0462]** Albumin fusion proteins and/or polynucleotides can be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

**[0463]** Albumin fusion proteins and/or polynucleotides of the invention are also suitably administered by sustained-release systems. Examples of sustained-release albumin fusion proteins and/or polynucleotides are administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion. Additional examples of sustained-release albumin fusion proteins and/or polynucleotides include suitable polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules), suitable hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

**[0464]** Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al., *Biopolymers* 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (Langer et al., *J. Biomed. Mater. Res.* 15:167-277 (1981), and Langer, *Chem. Tech.* 12:98-105 (1982)), ethylene vinyl acetate (Langer et al., *Id.*) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988).

**[0465]** Sustained-release albumin fusion proteins and/or polynucleotides also include liposomally entrapped albumin fusion proteins and/or polynucleotides of the invention (*see*

generally, Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317 - 327 and 353-365 (1989)). Liposomes containing the albumin fusion protein and/or polynucleotide are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci.(USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal Therapeutic.

[0466] In yet an additional embodiment, the albumin fusion proteins and/or polynucleotides of the invention are delivered by way of a pump (*see* Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)).

[0467] Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

[0468] For parenteral administration, in one embodiment, the albumin fusion protein and/or polynucleotide is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to the Therapeutic.

[0469] Generally, the formulations are prepared by contacting the albumin fusion protein and/or polynucleotide uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

[0470] The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate,



succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

**[0471]** The albumin fusion protein is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

**[0472]** Any pharmaceutical used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Albumin fusion proteins and/or polynucleotides generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

**[0473]** Albumin fusion proteins and/or polynucleotides ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous albumin fusion protein and/or polynucleotide solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized albumin fusion protein and/or polynucleotide using bacteriostatic Water-for-Injection.

**[0474]** In a specific and preferred embodiment, the Albumin fusion protein formulations comprises 0.01 M sodium phosphate, 0.15 mM sodium chloride, 0.16 micromole sodium octanoate/milligram of fusion protein, 15 micrograms/milliliter polysorbate 80, pH 7.2. In another specific and preferred embodiment, the Albumin fusion protein formulations consists 0.01 M sodium phosphate, 0.15 mM sodium chloride, 0.16 micromole sodium octanoate/milligram of fusion protein, 15 micrograms/milliliter polysorbate 80, pH 7.2. The pH and buffer are chosen to match physiological conditions and the salt is added as a tonicifier. Sodium octanoate has been chosen due to its reported ability to increase the thermal stability of the protein in solution. Finally, polysorbate has been

added as a generic surfactant, which lowers the surface tension of the solution and lowers non-specific adsorption of the albumin fusion protein to the container closure system.

[0475] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the albumin fusion proteins and/or polynucleotides of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the albumin fusion proteins and/or polynucleotides may be employed in conjunction with other therapeutic compounds.

[0476] The albumin fusion proteins and/or polynucleotides of the invention may be administered alone or in combination with adjuvants. Adjuvants that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG (e.g., THERACYS®), MPL and nonviable preparations of *Corynebacterium parvum*. In a specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are administered in combination with alum. In another specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are administered in combination with QS-21. Further adjuvants that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diphtheria, hepatitis A, hepatitis B, *Haemophilus influenzae* B, whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

**[0477]** The albumin fusion proteins and/or polynucleotides of the invention may be administered alone or in combination with other therapeutic agents. Albumin fusion protein and/or polynucleotide agents that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention, include but not limited to, chemotherapeutic agents, antibiotics, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, and/or therapeutic treatments described below. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

**[0478]** In one embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with an anticoagulant. Anticoagulants that may be administered with the compositions of the invention include, but are not limited to, heparin, low molecular weight heparin, warfarin sodium (e.g., COUMADIN®), dicumarol, 4-hydroxycoumarin, anisindione (e.g., MIRADON™), acenocoumarol (e.g., nicoumalone, SINTHROME™), indan-1,3-dione, phenprocoumon (e.g., MARCUMAR™), ethyl biscoumacetate (e.g., TROMEXAN™), and aspirin. In a specific embodiment, compositions of the invention are administered in combination with heparin and/or warfarin. In another specific embodiment, compositions of the invention are administered in combination with warfarin. In another specific embodiment, compositions of the invention are administered in combination with warfarin and aspirin. In another specific embodiment, compositions of the invention are administered in combination with heparin. In another specific embodiment, compositions of the invention are administered in combination with heparin and aspirin.

**[0479]** In another embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with thrombolytic drugs. Thrombolytic drugs that may be administered with the compositions of the invention include, but are not limited to, plasminogen, lys-plasminogen, alpha2-antiplasmin, streptokinase (e.g., KABIKINASE™), antirespace (e.g., EMINASE™), tissue plasminogen activator (t-PA, altevase, ACTIVASE™), urokinase (e.g., ABBOKINASE™), sauruplase, (Prourokinase, single chain urokinase), and aminocaproic acid (e.g., AMICAR™). In a specific embodiment,

compositions of the invention are administered in combination with tissue plasminogen activator and aspirin.

**[0480]** In another embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with antiplatelet drugs. Antiplatelet drugs that may be administered with the compositions of the invention include, but are not limited to, aspirin, dipyridamole (e.g., PERSANTINE™), and ticlopidine (e.g., TICLID™).

**[0481]** In specific embodiments, the use of anti-coagulants, thrombolytic and/or antiplatelet drugs in combination with albumin fusion proteins and/or polynucleotides of the invention is contemplated for the prevention, diagnosis, and/or treatment of thrombosis, arterial thrombosis, venous thrombosis, thromboembolism, pulmonary embolism, atherosclerosis, myocardial infarction, transient ischemic attack, unstable angina. In specific embodiments, the use of anticoagulants, thrombolytic drugs and/or antiplatelet drugs in combination with albumin fusion proteins and/or polynucleotides of the invention is contemplated for the prevention of occlusion of saphenous grafts, for reducing the risk of periprocedural thrombosis as might accompany angioplasty procedures, for reducing the risk of stroke in patients with atrial fibrillation including nonrheumatic atrial fibrillation, for reducing the risk of embolism associated with mechanical heart valves and or mitral valves disease. Other uses for the therapeutics of the invention, alone or in combination with antiplatelet, anticoagulant, and/or thrombolytic drugs, include, but are not limited to, the prevention of occlusions in extracorporeal devices (e.g., intravascular canulas, vascular access shunts in hemodialysis patients, hemodialysis machines, and cardiopulmonary bypass machines).

**[0482]** In certain embodiments, albumin fusion proteins and/or polynucleotides of the invention are administered in combination with antiretroviral agents, nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and/or protease inhibitors (PIs). NRTIs that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). NNRTIs that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the albumin fusion proteins

and/or polynucleotides of the invention, include, but are not limited to, CRIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with albumin fusion proteins and/or polynucleotides of the invention to treat AIDS and/or to prevent or treat HIV infection.

**[0483]** Additional NRTIs include LODENOSINE™ (F-ddA; an acid-stable adenosine NRTI; Triangle/Abbott); COVIRACIL™ (emtricitabine/FTC; structurally related to lamivudine (3TC) but with 3- to 10-fold greater activity *in vitro*; Triangle/Abbott); dOTC (BCH-10652, also structurally related to lamivudine but retains activity against a substantial proportion of lamivudine-resistant isolates; Biochem Pharma); Adefovir (refused approval for anti-HIV therapy by FDA; Gilead Sciences); PREVEON® (Adefovir Dipivoxil, the active prodrug of adefovir; its active form is PMEA-pp); TENOFOVIR™ (bis-POC PMPA, a PMPA prodrug; Gilead); DAPD/DXG (active metabolite of DAPD; Triangle/Abbott); D-D4FC (related to 3TC, with activity against AZT/3TC-resistant virus); GW420867X (Glaxo Wellcome); ZIAGEN™ (abacavir/159U89; Glaxo Wellcome Inc.); CS-87 (3'-azido-2',3'-dideoxyuridine; WO 99/66936); and S-acyl-2-thioethyl (SATE)-bearing prodrug forms of  $\beta$ -L-FD4C and  $\beta$ -L-FddC (WO 98/17281).

**[0484]** Additional NNRTIs include COACTINON™ (Emivirine/MKC-442, potent NNRTI of the HEPT class; Triangle/Abbott); CAPRAVIRINE™ (AG-1549/S-1153, a next generation NNRTI with activity against viruses containing the K103N mutation; Agouron); PNU-142721 (has 20- to 50-fold greater activity than its predecessor delavirdine and is active against K103N mutants; Pharmacia & Upjohn); DPC-961 and DPC-963 (second-generation derivatives of efavirenz, designed to be active against viruses with the K103N mutation; DuPont); GW-420867X (has 25-fold greater activity than HBY097 and is active against K103N mutants; Glaxo Wellcome); CALANOLIDE A (naturally occurring agent from the latex tree; active against viruses containing either or both the Y181C and K103N mutations); and Propolis (WO 99/49830).

**[0485]** Additional protease inhibitors include LOPINAVIR™ (ABT378/r; Abbott Laboratories); BMS-232632 (an azapeptide; Bristol-Myers Squibb); TIPRANAVIR™ (PNU-140690, a non-peptic dihydropyrone; Pharmacia & Upjohn); PD-178390 (a nonpeptidic dihydropyrone; Parke-Davis); BMS 232632 (an azapeptide; Bristol-Myers Squibb); L-

756,423 (an indinavir analog; Merck); DMP-450 (a cyclic urea compound; Avid & DuPont); AG-1776 (a peptidomimetic with *in vitro* activity against protease inhibitor-resistant viruses; Agouron); VX-175/GW-433908 (phosphate prodrug of amprenavir; Vertex & Glaxo Wellcome); CGP61755 (Ciba); and AGENERASE™ (amprenavir; Glaxo Wellcome Inc.).

**[0486]** Additional antiretroviral agents include fusion inhibitors/gp41 binders. Fusion inhibitors/gp41 binders include T-20 (a peptide from residues 643-678 of the HIV gp41 transmembrane protein ectodomain which binds to gp41 in its resting state and prevents transformation to the fusogenic state; Trimeris) and T-1249 (a second-generation fusion inhibitor; Trimeris).

**[0487]** Additional antiretroviral agents include fusion inhibitors/chemokine receptor antagonists. Fusion inhibitors/chemokine receptor antagonists include CXCR4 antagonists such as AMD 3100 (a bicyclam), SDF-1 and its analogs, and ALX40-4C (a cationic peptide), T22 (an 18 amino acid peptide; Trimeris) and the T22 analogs T134 and T140; CCR5 antagonists such as RANTES (9-68), AOP-RANTES, NNY-RANTES, and TAK-779; and CCR5/CXCR4 antagonists such as NSC 651016 (a distamycin analog). Also included are CCR2B, CCR3, and CCR6 antagonists. Chemokine receptor agonists such as RANTES, SDF-1, MIP-1 $\alpha$ , MIP-1 $\beta$ , etc., may also inhibit fusion.

**[0488]** Additional antiretroviral agents include integrase inhibitors. Integrase inhibitors include dicaffeoylquinic (DFQA) acids; L-chicoric acid (a dicaffeoyltartaric (DCTA) acid); quinalizarin (QLC) and related anthraquinones; ZINTEVIR™ (AR 177, an oligonucleotide that probably acts at cell surface rather than being a true integrase inhibitor; Arondex); and naphthols such as those disclosed in WO 98/50347.

**[0489]** Additional antiretroviral agents include hydroxyurea-like compounds such as BCX-34 (a purine nucleoside phosphorylase inhibitor; Biocryst); ribonucleotide reductase inhibitors such as DIDOX™ (Molecules for Health); inosine monophosphate dehydrogenase (IMPDH) inhibitors such as VX-497 (Vertex); and mycopholic acids such as CellCept (mycophenolate mofetil; Roche).

**[0490]** Additional antiretroviral agents include inhibitors of viral integrase, inhibitors of viral genome nuclear translocation such as arylene bis(methylketone) compounds; inhibitors of HIV entry such as AOP-RANTES, NNY-RANTES, RANTES-IgG fusion protein, soluble complexes of RANTES and glycosaminoglycans (GAG), and AMD-3100;

nucleocapsid zinc finger inhibitors such as dithiane compounds; targets of HIV Tat and Rev; and pharmacoenhancers such as ABT-378.

**[0491]** Other antiretroviral therapies and adjunct therapies include cytokines and lymphokines such as MIP-1 $\alpha$ , MIP-1 $\beta$ , SDF-1 $\alpha$ , IL-2, PROLEUKIN™ (aldesleukin/L2-7001; Chiron), IL-4, IL-10, IL-12, and IL-13; interferons such as IFN-alpha2a, IFN-alpha2b, or IFN-beta; antagonists of TNFs, NF $\kappa$ B, GM-CSF, M-CSF, and IL-10; agents that modulate immune activation such as cyclosporin and prednisone; vaccines such as Remune™ (HIV Immunogen), APL 400-003 (Apollon), recombinant gp120 and fragments, bivalent (B/E) recombinant envelope glycoprotein, rgp120CM235, MN rgp120, SF-2 rgp120, gp120/soluble CD4 complex, Delta JR-FL protein, branched synthetic peptide derived from discontinuous gp120 C3/C4 domain, fusion-competent immunogens, and Gag, Pol, Nef, and Tat vaccines; gene-based therapies such as genetic suppressor elements (GSEs; WO 98/54366), and intrakines (genetically modified CC chemokines targetted to the ER to block surface expression of newly synthesized CCR5 (Yang *et al.*, *PNAS* 94:11567-72 (1997); Chen *et al.*, *Nat. Med.* 3:1110-16 (1997)); antibodies such as the anti-CXCR4 antibody 12G5, the anti-CCR5 antibodies 2D7, 5C7, PA8, PA9, PA10, PA11, PA12, and PA14, the anti-CD4 antibodies Q4120 and RPA-T4, the anti-CCR3 antibody 7B11, the anti-gp120 antibodies 17b, 48d, 447-52D, 257-D, 268-D and 50.1, anti-Tat antibodies, anti-TNF- $\alpha$  antibodies, and monoclonal antibody 33A; aryl hydrocarbon (AH) receptor agonists and antagonists such as TCDD, 3,3',4,4',5-pentachlorobiphenyl, 3,3',4,4'-tetrachlorobiphenyl, and  $\alpha$ -naphthoflavone (WO 98/30213); and antioxidants such as  $\gamma$ -L-glutamyl-L-cysteine ethyl ester ( $\gamma$ -GCE; WO 99/56764).

**[0492]** In a further embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, remantidine, maxamine, or thymalfasin. Specifically, interferon albumin fusion protein can be administered in combination with any of these agents. Moreover, interferon alpha albumin fusion protein can also be administered with any of these agents, and preferably, interferon alpha 2a or 2b albumin fusion protein can be administered with any of these agents. Furthermore, interferon beta albumin fusion protein can also be administered with any of these agents. Additionally, any of the IFN hybrids albumin fusion proteins can be administered in combination with any of

these agents.

[0493] In a most preferred embodiment, interferon albumin fusion protein is administered in combination with ribavirin. In a further preferred embodiment, interferon alpha albumin fusion protein is administered in combination with ribavirin. In a further preferred embodiment, interferon alpha 2a albumin fusion protein is administered in combination with ribavirin. In a further preferred embodiment, interferon alpha 2b albumin fusion protein is administered in combination with ribavirin. In a further preferred embodiment, interferon beta albumin fusion protein is administered in combination with ribavirin. In a further preferred embodiment, hybrid interferon albumin fusion protein is administered in combination with ribavirin.

[0494] In other embodiments, albumin fusion proteins and/or polynucleotides of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™, PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ (sargramostim/GM-CSF). In a specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat or prevent an opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are used in any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat or prevent an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are used in any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat or prevent an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are used in any combination



with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat or prevent an opportunistic cytomegalovirus infection. In another specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to prophylactically treat or prevent an opportunistic fungal infection. In another specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are used in any combination with ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat or prevent an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are used in any combination with PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat or prevent an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat or prevent an opportunistic bacterial infection.

**[0495]** In a further embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, amoxicillin, beta-lactamases, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rapamycin, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamethoxazole, and vancomycin.

**[0496]** In other embodiments, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with immunestimulants. Immunestimulants that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, levamisole (e.g., ERGAMISOL™), isoprinosine (e.g. INOSIPLEX™), interferons (e.g. interferon alpha), and interleukins (e.g., IL-2).

**[0497]** In other embodiments, albumin fusion proteins and/or polynucleotides of the invention are administered in combination with immunosuppressive agents. Immunosuppressive agents that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, steroids,

cyclosporine, cyclosporine analogs, cyclophosphamide methylprednisone, prednisone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells. Other immunosuppressive agents that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, prednisolone, methotrexate, thalidomide, methoxsalen, rapamycin, leflunomide, mizoribine (BREDININ™), brequinar, deoxyspergualin, and azaspirane (SKF 105685), ORTHOCLONE OKT® 3 (muromonab-CD3), SANDIMMUNE™, NEORAL™, SANGDYA™ (cyclosporine), PROGRAF® (FK506, tacrolimus), CELLCEPT® (mycophenolate mofetil, of which the active metabolite is mycophenolic acid), IMURAN™ (azathioprine), glucocorticosteroids, adrenocortical steroids such as DELTASONE™ (prednisone) and HYDELTRASOL™ (prednisolone), FOLEX™ and MEXATE™ (methotrexate), OXSORALEN-ULTRA™ (methoxsalen) and RAPAMUNE™ (sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

**[0498]** In an additional embodiment, albumin fusion proteins and/or polynucleotides of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, ATGAM™ (antithymocyte globulin), and GAMIMUNE™. In a specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

**[0499]** In another embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered alone or as part of a combination therapy, either in vivo to patients or in vitro to cells, for the treatment of cancer. In a specific embodiment, the albumin fusion proteins, particularly IL-2-albumin fusions, are administered repeatedly during passive immunotherapy for cancer, such as adoptive cell transfer therapy for metastatic melanoma as described in Dudley *et al.* (Science Express, 19 September 2002., at [www.scienceexpress.org](http://www.scienceexpress.org), hereby incorporated by reference in its entirety).

**[0500]** In certain embodiments, the albumin fusion proteins and/or polynucleotides of the invention are administered alone or in combination with an anti-inflammatory agent.

Anti-inflammatory agents that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, corticosteroids (e.g. betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, and triamcinolone), nonsteroidal anti-inflammatory drugs (e.g., diclofenac, diflunisal, etodolac, fenoprofen, floctafenine, flurbiprofen, ibuprofen, indomethacin, ketoprofen, meclofenamate, mefenamic acid, meloxicam, nabumetone, naproxen, oxaprozin, phenylbutazone, piroxicam, sulindac, tenoxicam, tiaprofenic acid, and tolmetin.), as well as antihistamines, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

**[0501]** In an additional embodiment, the compositions of the invention are administered alone or in combination with an anti-angiogenic agent. Anti-angiogenic agents that may be administered with the compositions of the invention include, but are not limited to, Angiostatin (Entremed, Rockville, MD), Troponin-1 (Boston Life Sciences, Boston, MA), anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel (Taxol), Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, VEGI, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

**[0502]** Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

**[0503]** Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

**[0504]** Representative examples of tungsten and molybdenum complexes also include

oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

**[0505]** A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include, but are not limited to, platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., *Cancer Res.* 51:22-26, (1991)); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., *J. Bio. Chem.* 267:17321-17326, (1992)); Chymostatin (Tomkinson et al., *Biochem J.* 286:475-480, (1992)); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., *Nature* 348:555-557, (1990)); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, *J. Clin. Invest.* 79:1440-1446, (1987)); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., *J. Biol. Chem.* 262(4):1659-1664, (1987)); Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4- chloroanthronilic acid disodium or "CCA"; (Takeuchi et al., *Agents Actions* 36:312-316, (1992)); and metalloproteinase inhibitors such as BB94.

**[0506]** Additional anti-angiogenic factors that may also be utilized within the context of the present invention include Thalidomide, (Celgene, Warren, NJ); Angiostatic steroid; AGM-1470 (H. Brem and J. Folkman *J Pediatr. Surg.* 28:445-51 (1993)); an integrin alpha v beta 3 antagonist (C. Storgard et al., *J Clin. Invest.* 103:47-54 (1999)); carboxynaminolmidazole; Carboxyamidotriazole (CAI) (National Cancer Institute, Bethesda, MD); Conbretastatin A-4 (CA4P) (OXiGENE, Boston, MA); Squalamine (Magainin

Pharmaceuticals, Plymouth Meeting, PA); TNP-470, (Tap Pharmaceuticals, Deerfield, IL); ZD-0101 AstraZeneca (London, UK); APRA (CT2584); Benefin, Byrostatin-1 (SC339555); CGP-41251 (PKC 412); CM101; Dexrazoxane (ICRF187); DMXAA; Endostatin; Flavopridiol; Genestein; GTE; ImmTher; Iressa (ZD1839); Octreotide (Somatostatin); Panretin; Penacillamine; Photopoint; PI-88; Prinomastat (AG-3340) Purlitin; Suradista (FCE26644); Tamoxifen (Nolvadex); Tazarotene; Tetrathiomolybdate; Xeloda (Capecitabine); and 5-Fluorouracil.

**[0507]** Anti-angiogenic agents that may be administered in combination with the compounds of the invention may work through a variety of mechanisms including, but not limited to, inhibiting proteolysis of the extracellular matrix, blocking the function of endothelial cell-extracellular matrix adhesion molecules, by antagonizing the function of angiogenesis inducers such as growth factors, and inhibiting integrin receptors expressed on proliferating endothelial cells. Examples of anti-angiogenic inhibitors that interfere with extracellular matrix proteolysis and which may be administered in combination with the compositions of the invention include, but are not limited to, AG-3340 (Agouron, La Jolla, CA), BAY-12-9566 (Bayer, West Haven, CT), BMS-275291 (Bristol Myers Squibb, Princeton, NJ), CGS-27032A (Novartis, East Hanover, NJ), Marimastat (British Biotech, Oxford, UK), and Metastat (Aeterna, St-Foy, Quebec). Examples of anti-angiogenic inhibitors that act by blocking the function of endothelial cell-extracellular matrix adhesion molecules and which may be administered in combination with the compositions of the invention include, but are not limited to, EMD-121974 (Merck KgaA Darmstadt, Germany) and Vitaxin (Ixsys, La Jolla, CA/Medimmune, Gaithersburg, MD). Examples of anti-angiogenic agents that act by directly antagonizing or inhibiting angiogenesis inducers and which may be administered in combination with the compositions of the invention include, but are not limited to, Angiozyme (Ribozyme, Boulder, CO), Anti-VEGF antibody (Genentech, S. San Francisco, CA), PTK-787/ZK-225846 (Novartis, Basel, Switzerland), SU-101 (Sugen, S. San Francisco, CA), SU-5416 (Sugen/ Pharmacia Upjohn, Bridgewater, NJ), and SU-6668 (Sugen). Other anti-angiogenic agents act to indirectly inhibit angiogenesis. Examples of indirect inhibitors of angiogenesis which may be administered in combination with the compositions of the invention include, but are not limited to, IM-862 (Cytran, Kirkland, WA), Interferon-alpha, IL-12 (Roche, Nutley, NJ), and Pentosan polysulfate (Georgetown University, Washington, DC).

**[0508]** In particular embodiments, the use of compositions of the invention in

combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of an autoimmune disease, such as for example, an autoimmune disease described herein.

**[0509]** In a particular embodiment, the use of compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of arthritis. In a more particular embodiment, the use of compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of rheumatoid arthritis.

**[0510]** In another embodiment, the polynucleotides encoding a polypeptide of the present invention are administered in combination with an angiogenic protein, or polynucleotides encoding an angiogenic protein. Examples of angiogenic proteins that may be administered with the compositions of the invention include, but are not limited to, acidic and basic fibroblast growth factors, VEGF-1, VEGF-2, VEGF-3, epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin-like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide synthase.

**[0511]** In additional embodiments, compositions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to alkylating agents such as nitrogen mustards (for example, Mechlorethamine, cyclophosphamide, Cyclophosphamide Ifosfamide, Melphalan (L-sarcolysin), and Chlorambucil), ethylenimines and methylmelamines (for example, Hexamethylmelamine and Thiotepa), alkyl sulfonates (for example, Busulfan), nitrosoureas (for example, Carmustine (BCNU), Lomustine (CCNU), Semustine (methyl-CCNU), and Streptozocin (streptozotocin)), triazenes (for example, Dacarbazine (DTIC; dimethyltriazenoimidazolecarboxamide)), folic acid analogs (for example, Methotrexate (amethopterin)), pyrimidine analogs (for example, Fluorouracil (5-fluorouracil; 5-FU), Floxuridine (fluorodeoxyuridine; FudR), and Cytarabine (cytosine arabinoside)), purine analogs and related inhibitors (for example, Mercaptopurine (6-mercaptopurine; 6-MP), Thioguanine (6-thioguanine; TG), and Pentostatin (2'-deoxycoformycin)), vinca alkaloids (for example, Vinblastine (VLB, vinblastine sulfate)) and Vincristine (vincristine sulfate)), epipodophyllotoxins (for example, Etoposide and Teniposide), antibiotics (for example,

Dactinomycin (actinomycin D), Daunorubicin (daunomycin; rubidomycin), Doxorubicin, Bleomycin, Plicamycin (mithramycin), and Mitomycin (mitomycin C), enzymes (for example, L-Asparaginase), biological response modifiers (for example, Interferon-alpha and interferon-alpha-2b), platinum coordination compounds (for example, Cisplatin (cis-DDP) and Carboplatin), anthracenedione (Mitoxantrone), substituted ureas (for example, Hydroxyurea), methylhydrazine derivatives (for example, Procarbazine (N-methylhydrazine; MIH), adrenocorticosteroids (for example, Prednisone), progestins (for example, Hydroxyprogesterone caproate, Medroxyprogesterone, Medroxyprogesterone acetate, and Megestrol acetate), estrogens (for example, Diethylstilbestrol (DES), Diethylstilbestrol diphosphate, Estradiol, and Ethinyl estradiol), antiestrogens (for example, Tamoxifen), androgens (Testosterone propionate, and Fluoxymesterone), antiandrogens (for example, Flutamide), gonadotropin-releasing hormone analogs (for example, Leuprolide), other hormones and hormone analogs (for example, methyltestosterone, estramustine, estramustine phosphate sodium, chlorotrianisene, and testolactone), and others (for example, dicarbazine, glutamic acid, and mitotane).

**[0512]** In one embodiment, the compositions of the invention are administered in combination with one or more of the following drugs: infliximab (also known as Remicade™ Centocor, Inc.), Trocade (Roche, RO-32-3555), Leflunomide (also known as Arava™ from Hoechst Marion Roussel), Kineret™ (an IL-1 Receptor antagonist also known as Anakinra from Amgen, Inc.)

**[0513]** In a specific embodiment, compositions of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or combination of one or more of the components of CHOP. In one embodiment, the compositions of the invention are administered in combination with anti-CD20 antibodies, human monoclonal anti-CD20 antibodies. In another embodiment, the compositions of the invention are administered in combination with anti-CD20 antibodies and CHOP, or anti-CD20 antibodies and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. In a specific embodiment, compositions of the invention are administered in combination with Rituximab. In a further embodiment, compositions of the invention are administered with Rituximab and CHOP, or Rituximab and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. In a specific embodiment, compositions of the invention are administered in combination with tositumomab. In a further embodiment, compositions of the invention

are administered with tositumomab and CHOP, or tositumomab and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. The anti-CD20 antibodies may optionally be associated with radioisotopes, toxins or cytotoxic prodrugs.

**[0514]** In another specific embodiment, the compositions of the invention are administered in combination Zevalin™. In a further embodiment, compositions of the invention are administered with Zevalin™ and CHOP, or Zevalin™ and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. Zevalin™ may be associated with one or more radisotopes. Particularly preferred isotopes are <sup>90</sup>Y and <sup>111</sup>In.

**[0515]** In an additional embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with cytokines. Cytokines that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha. In another embodiment, albumin fusion proteins and/or polynucleotides of the invention may be administered with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21.

**[0516]** In one embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), endokine-alpha (International Publication No. WO 98/07880), OPG, and neutrokin-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TRANK, TR9 (International Publication No. WO 98/56892), TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.



**[0517]** In an additional embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with angiogenic proteins. Angiogenic proteins that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, Glioma Derived Growth Factor (GDGF), as disclosed in European Patent Number EP-399816; Platelet Derived Growth Factor-A (PDGF-A), as disclosed in European Patent Number EP-682110; Platelet Derived Growth Factor-B (PDGF-B), as disclosed in European Patent Number EP-282317; Placental Growth Factor (PlGF), as disclosed in International Publication Number WO 92/06194; Placental Growth Factor-2 (PlGF-2), as disclosed in Hauser et al., Growth Factors, 4:259-268 (1993); Vascular Endothelial Growth Factor (VEGF), as disclosed in International Publication Number WO 90/13649; Vascular Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-506477; Vascular Endothelial Growth Factor-2 (VEGF-2), as disclosed in International Publication Number WO 96/39515; Vascular Endothelial Growth Factor B (VEGF-3); Vascular Endothelial Growth Factor B-186 (VEGF-B186), as disclosed in International Publication Number WO 96/26736; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/02543; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/07832; and Vascular Endothelial Growth Factor-E (VEGF-E), as disclosed in German Patent Number DE19639601. The above mentioned references are herein incorporated by reference in their entireties.

**[0518]** In an additional embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

**[0519]** In an additional embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, granulocyte macrophage colony stimulating factor (GM-CSF) (sargramostim, LEUKINE™, PROKINE™), granulocyte colony stimulating factor (G-CSF) (filgrastim, NEUPOGEN™), macrophage colony stimulating factor (M-CSF, CSF-1) erythropoietin (epoetin alfa,

EPOGEN™, PROCRT™, stem cell factor (SCF, c-kit ligand, steel factor), megakaryocyte colony stimulating factor, PIXY321 (a GMCSF/IL-3 fusion protein), interleukins, especially any one or more of IL-1 through IL-12, interferon-gamma, or thrombopoietin.

**[0520]** In certain embodiments, albumin fusion proteins and/or polynucleotides of the present invention are administered in combination with adrenergic blockers, such as, for example, acebutolol, atenolol, betaxolol, bisoprolol, carteolol, labetalol, metoprolol, nadolol, oxprenolol, penbutolol, pindolol, propranolol, sotalol, and timolol.

**[0521]** In another embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with an antiarrhythmic drug (e.g., adenosine, amidoarone, bretylium, digitalis, digoxin, digitoxin, diltiazem, disopyramide, esmolol, flecainide, lidocaine, mexiletine, moricizine, phenytoin, procainamide, N-acetyl procainamide, propafenone, propranolol, quinidine, sotalol, tocainide, and verapamil).

**[0522]** In another embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with diuretic agents, such as carbonic anhydrase-inhibiting agents (e.g., acetazolamide, dichlorphenamide, and methazolamide), osmotic diuretics (e.g., glycerin, isosorbide, mannitol, and urea), diuretics that inhibit Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> symport (e.g., furosemide, bumetanide, azosemide, piretanide, triparamide, ethacrynic acid, muzolimine, and torsemide), thiazide and thiazide-like diuretics (e.g., bendroflumethiazide, benzthiazide, chlorothiazide, hydrochlorothiazide, hydroflumethiazide, methyclothiazide, polythiazide, trichormethiazide, chlorthalidone, indapamide, metolazone, and quinethazone), potassium sparing diuretics (e.g., amiloride and triamterene), and mineralcorticoid receptor antagonists (e.g., spironolactone, canrenone, and potassium canrenoate).

**[0523]** In one embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with treatments for endocrine and/or hormone imbalance disorders. Treatments for endocrine and/or hormone imbalance disorders include, but are not limited to, <sup>127</sup>I, radioactive isotopes of iodine such as <sup>131</sup>I and <sup>123</sup>I; recombinant growth hormone, such as HUMATROPE™ (recombinant somatotropin); growth hormone analogs such as PROTROPIN™ (somatrem); dopamine agonists such as PARLODEL™ (bromocriptine); somatostatin analogs such as SANDOSTATIN™ (octreotide); gonadotropin preparations such as PREGNYL™, A.P.L.™ and PROFASI™ (chorionic gonadotropin (CG)), PERGONAL™ (menotropins), and METRODIN™ (urofollitropin (uFSH)); synthetic human

gonadotropin releasing hormone preparations such as FACTREL™ and LUTREPULSE™ (gonadorelin hydrochloride); synthetic gonadotropin agonists such as LUPRON™ (leuprolide acetate), SUPPRELIN™ (histrelin acetate), SYNAREL™ (nafarelin acetate), and ZOLADEX™ (goserelin acetate); synthetic preparations of thyrotropin-releasing hormone such as RELEFACT TRH™ and THYPINONE™ (protirelin); recombinant human TSH such as THYROGEN™; synthetic preparations of the sodium salts of the natural isomers of thyroid hormones such as L-T<sub>4</sub>™, SYNTHROID™ and LEVOTHROID™ (levothyroxine sodium), L-T<sub>3</sub>™, CYTOMEL™ and TRIOSTAT™ (liothyroine sodium), and THYROLAR™ (liotrix); antithyroid compounds such as 6-*n*-propylthiouracil (propylthiouracil), 1-methyl-2-mercaptoimidazole and TAPAZOLE™ (methimazole), NEO-MERCAZOLE™ (carbimazole); beta-adrenergic receptor antagonists such as propranolol and esmolol; Ca<sup>2+</sup> channel blockers; dexamethasone and iodinated radiological contrast agents such as TELEPAQUE™ (iopanoic acid) and ORAGRAFIN™ (sodium ipodate).

**[0524]** Additional treatments for endocrine and/or hormone imbalance disorders include, but are not limited to, estrogens or conjugated estrogens such as ESTRACE™ (estradiol), ESTINYL™ (ethinyl estradiol), PREMARIN™, ESTRATAB™, ORTHO-EST™, OGEN™ and estropipate (estrone), ESTROVIS™ (quinestrol), ESTRADERM™ (estradiol), DELESTROGEN™ and VALERGEN™ (estradiol valerate), DEPO-ESTRADIOL CYPIONATE™ and ESTROJECT LA™ (estradiol cypionate); antiestrogens such as NOLVADEX™ (tamoxifen), SEROPHENE™ and CLOMID™ (clomiphene); progestins such as DURALUTIN™ (hydroxyprogesterone caproate), MPA™ and DEPO-PROVERA™ (medroxyprogesterone acetate), PROVERA™ and CYCRIN™ (MPA), MEGACE™ (megestrol acetate), NORLUTIN™ (norethindrone), and NORLUTATE™ and AYGESTIN™ (norethindrone acetate); progesterone implants such as NORPLANT SYSTEM™ (subdermal implants of norgestrel); antiprogestins such as RU 486™ (mifepristone); hormonal contraceptives such as ENOVID™ (norethynodrel plus mestranol), PROGESTASERT™ (intrauterine device that releases progesterone), LOESTRIN™, BREVICON™, MODICON™, GENORA™, NELONA™, NORINYL™, OVACON-35™ and OVACON-50™ (ethinyl estradiol/norethindrone), LEVLEN™, NORDETTE™, TRI-LEVLEN™ and TRIPHASIL-21™ (ethinyl estradiol/levonorgestrel) LO/OVRAL™ and OVRAL™ (ethinyl estradiol/norgestrel), DEMULEN™ (ethinyl estradiol/ethynodiol diacetate), NORINYL™, ORTHO-NOVUM™,

NORETHIN™, GENORA™, and NELOVA™ (norethindrone/mestranol), DESOGEN™ and ORTHO-CEPT™ (ethinyl estradiol/desogestrel), ORTHO-CYCLEN™ and ORTHO-TRICYCLEN™ (ethinyl estradiol/norgestimate), MICRONOR™ and NOR-QD™ (norethindrone), and OVRETTE™ (norgestrel).

**[0525]** Additional treatments for endocrine and/or hormone imbalance disorders include, but are not limited to, testosterone esters such as methenolone acetate and testosterone undecanoate; parenteral and oral androgens such as TESTOJECT-50™ (testosterone), TESTEX™ (testosterone propionate), DELATESTRYL™ (testosterone enanthate), DEPO-TESTOSTERONE™ (testosterone cypionate), DANOCRINE™ (danazol), HALOTESTIN™ (fluoxymesterone), ORETON METHYL™, TESTRED™ and VIRILON™ (methyltestosterone), and OXANDRIN™ (oxandrolone); testosterone transdermal systems such as TESTODERM™; androgen receptor antagonist and 5-alpha-reductase inhibitors such as ANDROCUR™ (cyproterone acetate), EULEXIN™ (flutamide), and PROSCAR™ (finasteride); adrenocorticotrophic hormone preparations such as CORTROSYN™ (cosyntropin); adrenocortical steroids and their synthetic analogs such as ACLOVATE™ (alclometasone dipropionate), CYCLOCORT™ (amcinonide), BECLOVENT™ and VANCERIL™ (beclomethasone dipropionate), CELESTONE™ (betamethasone), BENISONE™ and UTICORT™ (betamethasone benzoate), DIPROSONE™ (betamethasone dipropionate), CELESTONE PHOSPHATE™ (betamethasone sodium phosphate), CELESTONE SOLUSPAN™ (betamethasone sodium phosphate and acetate), BETA-VAL™ and VALISONE™ (betamethasone valerate), TEMOVATE™ (clobetasol propionate), CLODERM™ (clocortolone pivalate), CORTEF™ and HYDROCORTONE™ (cortisol (hydrocortisone)), HYDROCORTONE ACETATE™ (cortisol (hydrocortisone) acetate), LOCOID™ (cortisol (hydrocortisone) butyrate), HYDROCORTONE PHOSPHATE™ (cortisol (hydrocortisone) sodium phosphate), A-HYDROCORT™ and SOLU CORTEF™ (cortisol (hydrocortisone) sodium succinate), WESTCORT™ (cortisol (hydrocortisone) valerate), CORTISONE ACETATE™ (cortisone acetate), DESOWEN™ and TRIDESILON™ (desonide), TOPICORT™ (desoximetasone), DECADRON™ (dexamethasone), DECADRON LA™ (dexamethasone acetate), DECADRON PHOSPHATE™ and HEXADROL PHOSPHATE™ (dexamethasone sodium phosphate), FLORONE™ and MAXIFLOR™ (diflorasone diacetate), FLORINEF ACETATE™

(fludrocortisone acetate), AEROBID™ and NASALIDE™ (flunisolide), FLUONID™ and SYNALAR™ (fluocinolone acetonide), LIDEX™ (fluocinonide), FLUOR-OP™ and FML™ (fluorometholone), CORDRAN™ (flurandrenolide), HALOG™ (halcinonide), HMS LIZUIFILM™ (medrysone), MEDROL™ (methylprednisolone), DEPO-MEDROL™ and MEDROL ACETATE™ (methylprednisone acetate), A-METHAPRED™ and SOLUMEDROL™ (methylprednisolone sodium succinate), ELOCON™ (mometasone furoate), HALDRONE™ (paramethasone acetate), DELTA-CORTEF™ (prednisolone), ECONOPRED™ (prednisolone acetate), HYDELTRASOL™ (prednisolone sodium phosphate), HYDELTRA-T.B.A™ (prednisolone tebutate), DELTASONE™ (prednisone), ARISTOCORT™ and KENACORT™ (triamcinolone), KENALOG™ (triamcinolone acetonide), ARISTOCORT™ and KENACORT DIACETATE™ (triamcinolone diacetate), and ARISTOSPAN™ (triamcinolone hexacetonide); inhibitors of biosynthesis and action of adrenocortical steroids such as CYTADREN™ (aminoglutethimide), NIZORAL™ (ketoconazole), MODRASTANE™ (trilostane), and METOPIRONE™ (metyrapone); bovine, porcine or human insulin or mixtures thereof; insulin analogs; recombinant human insulin such as HUMULIN™ and NOVOLIN™; oral hypoglycemic agents such as ORAMIDE™ and ORINASE™ (tolbutamide), DIABINESE™ (chlorpropamide), TOLAMIDE™ and TOLINASE™ (tolazamide), DYMELOR™ (acetoexamide), glibenclamide, MICRONASE™, DIBETA™ and GLYNASE™ (glyburide), GLUCOTROL™ (glipizide), and DIAMICRON™ (gliclazide), GLUCOPHAGE™ (metformin), ciglitazone, pioglitazone, and alpha-glucosidase inhibitors; bovine or porcine glucagon; somatostatins such as SANDOSTATIN™ (octreotide); and diazoxides such as PROGLYCEM™ (diazoxide).

**[0526]** In one embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with treatments for uterine motility disorders. Treatments for uterine motility disorders include, but are not limited to, estrogen drugs such as conjugated estrogens (e.g., PREMARIN® and ESTRATAB®), estradiols (e.g., CLIMARA® and ALORA®), estropipate, and chlorotrianisene; progestin drugs (e.g., AMEN® (medroxyprogesterone), MICRONOR® (norethidrone acetate), PROMETRIUM® progesterone, and megestrol acetate); and estrogen/progesterone combination therapies such as, for example, conjugated estrogens/medroxyprogesterone (e.g., PREMPRO™ and PREMPHASE®) and norethindrone acetate/ethinyl estsradiol (e.g., FEMHRT™).

**[0527]** In an additional embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with drugs effective in treating iron deficiency and hypochromic anemias, including but not limited to, ferrous sulfate (iron sulfate, FEOSOL™), ferrous fumarate (e.g., FEOSTAT™), ferrous gluconate (e.g., FERGON™), polysaccharide-iron complex (e.g., NIFEREX™), iron dextran injection (e.g., INFED™), cupric sulfate, pyroxidine, riboflavin, Vitamin B<sub>12</sub>, cyanocobalamin injection (e.g., REDISOL™, RUBRAMIN PC™), hydroxocobalamin, folic acid (e.g., FOLVITE™), leucovorin (folinic acid, 5-CHOH4PteGlu, citrovorum factor) or WELLCOVORIN (Calcium salt of leucovorin), transferrin or ferritin.

**[0528]** In certain embodiments, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with agents used to treat psychiatric disorders. Psychiatric drugs that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, antipsychotic agents (e.g., chlorpromazine, chlorprothixene, clozapine, fluphenazine, haloperidol, loxapine, mesoridazine, molindone, olanzapine, perphenazine, pimozide, quetiapine, risperidone, thioridazine, thiothixene, trifluoperazine, and triflupromazine), antimanic agents (e.g., carbamazepine, divalproex sodium, lithium carbonate, and lithium citrate), antidepressants (e.g., amitriptyline, amoxapine, bupropion, citalopram, clomipramine, desipramine, doxepin, fluvoxamine, fluoxetine, imipramine, isocarboxazid, maprotiline, mirtazapine, nefazodone, nortriptyline, paroxetine, phenelzine, protriptyline, sertraline, tranylcypromine, trazodone, trimipramine, and venlafaxine), antianxiety agents (e.g., alprazolam, buspirone, chlordiazepoxide, clorazepate, diazepam, halazepam, lorazepam, oxazepam, and prazepam), and stimulants (e.g., d-amphetamine, methylphenidate, and pemoline).

**[0529]** In other embodiments, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with agents used to treat neurological disorders. Neurological agents that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, antiepileptic agents (e.g., carbamazepine, clonazepam, ethosuximide, phenobarbital, phenytoin, primidone, valproic acid, divalproex sodium, felbamate, gabapentin, lamotrigine, levetiracetam, oxcarbazepine, tiagabine, topiramate, zonisamide, diazepam, lorazepam, and clonazepam), antiparkinsonian agents (e.g., levodopa/carbidopa, selegiline, amantidine, bromocriptine, pergolide, ropinirole, pramipexole, benzotropine; biperiden; ethopropazine; procyclidine; trihexyphenidyl, tolcapone), and ALS therapeutics (e.g. riluzole).

**[0530]** In another embodiment, albumin fusion proteins and/or polynucleotides of the invention are administered in combination with vasodilating agents and/or calcium channel blocking agents. Vasodilating agents that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, Angiotensin Converting Enzyme (ACE) inhibitors (e.g., papaverine, isoxsuprine, benazepril, captopril, cilazapril, enalapril, enalaprilat, fosinopril, lisinopril, moexipril, perindopril, quinapril, ramipril, spirapril, trandolapril, and nylidrin), and nitrates (e.g., isosorbide dinitrate, isosorbide mononitrate, and nitroglycerin). Examples of calcium channel blocking agents that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to amlodipine, bepridil, diltiazem, felodipine, flunarizine, isradipine, nicardipine, nifedipine, nimodipine, and verapamil.

**[0531]** In certain embodiments, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with treatments for gastrointestinal disorders. Treatments for gastrointestinal disorders that may be administered with the albumin fusion protein and/or polynucleotide of the invention include, but are not limited to, H<sub>2</sub> histamine receptor antagonists (e.g., TAGAMET<sup>TM</sup> (cimetidine), ZANTAC<sup>TM</sup> (ranitidine), PEPCID<sup>TM</sup> (famotidine), and AXID<sup>TM</sup> (nizatidine)); inhibitors of H<sup>+</sup>, K<sup>+</sup> ATPase (e.g., PREVACID<sup>TM</sup> (lansoprazole) and PRILOSEC<sup>TM</sup> (omeprazole)); Bismuth compounds (e.g., PEPTO-BISMOL<sup>TM</sup> (bismuth subsalicylate) and DE-NOL<sup>TM</sup> (bismuth subcitrate)); various antacids; sucralfate; prostaglandin analogs (e.g. CYTOTEC<sup>TM</sup> (misoprostol)); muscarinic cholinergic antagonists; laxatives (e.g., surfactant laxatives, stimulant laxatives, saline and osmotic laxatives); antidiarrheal agents (e.g., LOMOTIL<sup>TM</sup> (diphenoxylate), MOTOFEN<sup>TM</sup> (diphenoxin), and IMODIUM<sup>TM</sup> (loperamide hydrochloride)), synthetic analogs of somatostatin such as SANDOSTATIN<sup>TM</sup> (octreotide), antiemetic agents (e.g., ZOFRAN<sup>TM</sup> (ondansetron), KYTRIL<sup>TM</sup> (granisetron hydrochloride), tropisetron, dolasetron, metoclopramide, chlorpromazine, perphenazine, prochlorperazine, promethazine, thiethylperazine, triflupromazine, domperidone, haloperidol, droperidol, trimethobenzamide, dexamethasone, methylprednisolone, dronabinol, and nabilone); D2 antagonists (e.g., metoclopramide, trimethobenzamide and chlorpromazine); bile salts; chenodeoxycholic acid; ursodeoxycholic acid; and pancreatic enzyme preparations such as pancreatin and pancrelipase.

**[0532]** In additional embodiments, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with other therapeutic or prophylactic

regimens, such as, for example, radiation therapy.

[0533] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions comprising albumin fusion proteins of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

### ***Gene Therapy***

[0534] Constructs encoding albumin fusion proteins of the invention can be used as a part of a gene therapy protocol to deliver therapeutically effective doses of the albumin fusion protein. A preferred approach for *in vivo* introduction of nucleic acid into a cell is by use of a viral vector containing nucleic acid, encoding an albumin fusion protein of the invention. Infection of cells with a viral vector has the advantage that a large proportion of the targeted cells can receive the nucleic acid. Additionally, molecules encoded within the viral vector, *e.g.*, by a cDNA contained in the viral vector, are expressed efficiently in cells which have taken up viral vector nucleic acid.

[0535] Retrovirus vectors and adeno-associated virus vectors can be used as a recombinant gene delivery system for the transfer of exogenous nucleic acid molecules encoding albumin fusion proteins *in vivo*. These vectors provide efficient delivery of nucleic acids into cells, and the transferred nucleic acids are stably integrated into the chromosomal DNA of the host. The development of specialized cell lines (termed "packaging cells") which produce only replication-defective retroviruses has increased the utility of retroviruses for gene therapy, and defective retroviruses are characterized for use in gene transfer for gene therapy purposes (for a review see Miller, A.D. (1990) *Blood* 76:27 1). A replication defective retrovirus can be packaged into virions which can be used to infect a target cell through the use of a helper virus by standard techniques. Protocols for producing recombinant retroviruses and for infecting cells *in vitro* or *in vivo* with such viruses can be found in Current Protocols in Molecular Biology, Ausubel, F.M. *et al.*, (eds.) Greene Publishing Associates, (1989), Sections 9.10-9.14 and other standard laboratory manuals.

[0536] Another viral gene delivery system useful in the present invention uses adenovirus-derived vectors. The genome of an adenovirus can be manipulated such that it



encodes and expresses a gene product of interest but is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. See, for example, Berkner *et al.*, *BioTechniques* 6:616 (1988); Rosenfeld *et al.*, *Science* 252:431-434 (1991); and Rosenfeld *et al.*, *Cell* 68:143-155 (1992). Suitable adenoviral vectors derived from the adenovirus strain Ad type 5 d1324 or other strains of adenovirus (*e.g.*, Ad2, Ad3, Ad7 etc.) are known to those skilled in the art. Recombinant adenoviruses can be advantageous in certain circumstances in that they are not capable of infecting nondividing cells and can be used to infect a wide variety of cell types, including epithelial cells (Rosenfeld *et al.*, (1992) cited *supra*). Furthermore, the virus particle is relatively stable and amenable to purification and concentration, and as above, can be modified so as to affect the spectrum of infectivity. Additionally, introduced adenoviral DNA (and foreign DNA contained therein) is not integrated into the genome of a host cell but remains episomal, thereby avoiding potential problems that can occur as a result of insertional mutagenesis in situations where introduced DNA becomes integrated into the host genome (*e.g.*, retroviral DNA). Moreover, the carrying capacity of the adenoviral genome for foreign DNA is large (up to 8 kilobases) relative to other gene delivery vectors (Berkner *et al.*, cited *supra*; Haj-Ahmand *et al.*, *J. Virol.* 57:267 (1986)).

**[0537]** In another embodiment, non-viral gene delivery systems of the present invention rely on endocytic pathways for the uptake of the subject nucleotide molecule by the targeted cell. Exemplary gene delivery systems of this type include liposomal derived systems, poly-lysine conjugates, and artificial viral envelopes. In a representative embodiment, a nucleic acid molecule encoding an albumin fusion protein of the invention can be entrapped in liposomes bearing positive charges on their surface (*e.g.*, lipofectins) and (optionally) which are tagged with antibodies against cell surface antigens of the target tissue (Mizuno *et al.* (1992) *No Shinkei Geka* 20:547-5 5 1; PCT publication W091/06309; Japanese patent application 1047381; and European patent publication EP-A-43075).

**[0538]** Gene delivery systems for a gene encoding an albumin fusion protein of the invention can be introduced into a patient by any of a number of methods. For instance, a pharmaceutical preparation of the gene delivery system can be introduced systemically, *e.g.* by intravenous injection, and specific transduction of the protein in the target cells occurs predominantly from specificity of transfection provided by the gene delivery vehicle, cell-type or tissue-type expression due to the transcriptional regulatory sequences controlling expression of the receptor gene, or a combination thereof. In other embodiments, initial delivery of the recombinant gene is more limited with introduction into the animal being

quite localized. For example, the gene delivery vehicle can be introduced by catheter (see U.S. Patent 5,328,470) or by Stereotactic injection (*e.g.* Chen *et al.* (1994) *PNAS* 91: 3 054-3 05 7). The pharmaceutical preparation of the gene therapy construct can consist essentially of the gene delivery system in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Where the albumin fusion protein can be produced intact from recombinant cells, *e.g.* retroviral vectors, the pharmaceutical preparation can comprise one or more cells which produce the albumin fusion protein.

### ***Additional Gene Therapy Methods***

**[0539]** Also encompassed by the invention are gene therapy methods for treating or preventing disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of an albumin fusion protein of the invention. This method requires a polynucleotide which codes for an albumin fusion protein of the present invention operatively linked to a promoter and any other genetic elements necessary for the expression of the fusion protein by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein incorporated by reference.

**[0540]** Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a polynucleotide encoding an albumin fusion protein of the present invention *ex vivo*, with the engineered cells then being provided to a patient to be treated with the fusion protein of the present invention. Such methods are well-known in the art. For example, see Beldegrun, A., *et al.*, *J. Natl. Cancer Inst.* 85: 207-216 (1993); Ferrantini, M. *et al.*, *Cancer Research* 53: 1107-1112 (1993); Ferrantini, M. *et al.*, *J. Immunology* 153: 4604-4615 (1994); Kaido, T., *et al.*, *Int. J. Cancer* 60: 221-229 (1995); Ogura, H., *et al.*, *Cancer Research* 50: 5102-5106 (1990); Santodonato, L., *et al.*, *Human Gene Therapy* 7:1-10 (1996); Santodonato, L., *et al.*, *Gene Therapy* 4:1246-1255 (1997); and Zhang, J.-F. *et al.*, *Cancer Gene Therapy* 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells which are engineered are arterial cells. The arterial cells may be reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

**[0541]** As discussed in more detail below, the polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, and the like).

The polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

**[0542]** In one embodiment, polynucleotides encoding the albumin fusion proteins of the present invention is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, polynucleotides encoding the albumin fusion proteins of the present invention can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and 5,580,859, which are herein incorporated by reference.

**[0543]** The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.

**[0544]** Any strong promoter known to those skilled in the art can be used for driving the expression of the polynucleotide sequence. Suitable promoters include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the  $\beta$ -actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the gene corresponding to the Therapeutic protein portion of the albumin fusion proteins of the invention.

**[0545]** Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

**[0546]** The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular, fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

**[0547]** For the naked nucleic acid sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

**[0548]** The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked DNA constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

**[0549]** The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

**[0550]** The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

**[0551]** In certain embodiments, the polynucleotide constructs are complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA (1987) 84:7413-7416, which is herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA (1989) 86:6077-6081, which is herein incorporated by reference); and purified transcription factors (Debs et al., J. Biol. Chem. (1990) 265:10189-10192, which is herein incorporated by reference), in functional form.

**[0552]** Cationic liposomes are readily available. For example, N[1-2,3-dioleoyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl. Acad. Sci. USA (1987) 84:7413-7416, which is herein incorporated by reference). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).

**[0553]** Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication No. WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., P. Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

**[0554]** Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl, choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

[0555] For example, commercially dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15EC. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to produce unilamellar vesicles of discrete size. Other methods are known and available to those of skill in the art.

[0556] The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred. The various liposome-nucleic acid complexes are prepared using methods well known in the art. See, e.g., Straubinger et al., *Methods of Immunology* (1983), 101:512-527, which is herein incorporated by reference. For example, MLVs containing nucleic acid can be prepared by depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated. SUVs are prepared by extended sonication of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and then the preformed liposomes are mixed directly with the DNA. The liposome and DNA form a very stable complex due to binding of the positively charged liposomes to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods include  $\text{Ca}^{2+}$ -EDTA chelation (Papahadjopoulos et al., *Biochim. Biophys. Acta* (1975) 394:483; Wilson et al., *Cell* 17:77 (1979)); ether injection (Deamer, D. and Bangham, A., *Biochim. Biophys. Acta* 443:629 (1976); Ostro et al., *Biochem. Biophys. Res. Commun.* 76:836 (1977); Fraley et al., *Proc. Natl. Acad. Sci. USA* 76:3348 (1979)); detergent dialysis (Enoch, H. and Strittmatter, P., *Proc. Natl. Acad. Sci. USA* 76:145 (1979)); and reverse-phase evaporation (REV) (Fraley et al., *J. Biol. Chem.* 255:10431 (1980); Szoka, F. and Papahadjopoulos, D., *Proc. Natl. Acad.*

Sci. USA 75:145 (1978); Schaefer-Ridder et al., Science 215:166 (1982)), which are herein incorporated by reference.

[0557] Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ration will be from about 5:1 to about 1:5. More preferably, the ration will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

[0558] U.S. Patent No. 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no. WO 94/9469 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no. WO 94/9469 provide methods for delivering DNA-cationic lipid complexes to mammals.

[0559] In certain embodiments, cells are engineered, *ex vivo* or *in vivo*, using a retroviral particle containing RNA which comprises a sequence encoding an albumin fusion protein of the present invention. Retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

[0560] The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO<sub>4</sub> precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

[0561] The producer cell line generates infectious retroviral vector particles which include polynucleotide encoding an albumin fusion protein of the present invention. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express a fusion protin of the present

invention.

**[0562]** In certain other embodiments, cells are engineered, *ex vivo* or *in vivo*, with polynucleotide contained in an adenovirus vector. Adenovirus can be manipulated such that it encodes and expresses fusion protein of the present invention, and at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the host cell chromosome, thereby alleviating concerns about insertional mutagenesis. Furthermore, adenoviruses have been used as live enteric vaccines for many years with an excellent safety profile (Schwartz et al. Am. Rev. Respir. Dis.109:233-238 (1974)). Finally, adenovirus mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld, M. A. et al. (1991) Science 252:431-434; Rosenfeld et al., (1992) Cell 68:143-155). Furthermore, extensive studies to attempt to establish adenovirus as a causative agent in human cancer were uniformly negative (Green, M. et al. (1979) Proc. Natl. Acad. Sci. USA 76:6606).

**[0563]** Suitable adenoviral vectors useful in the present invention are described, for example, in Kozarsky and Wilson, Curr. Opin. Genet. Devel. 3:499-503 (1993); Rosenfeld et al., Cell 68:143-155 (1992); Engelhardt et al., Human Genet. Ther. 4:759-769 (1993); Yang et al., Nature Genet. 7:362-369 (1994); Wilson et al., Nature 365:691-692 (1993); and U.S. Patent No. 5,652,224, which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the E1 region of adenovirus and constitutively express Ela and Elb, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to Ad2, other varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.

**[0564]** Preferably, the adenoviruses used in the present invention are replication deficient. Replication deficient adenoviruses require the aid of a helper virus and/or packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest which is operably linked to a promoter, but cannot replicate in most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: E1a, E1b, E3, E4, E2a, or L1 through L5.

**[0565]** In certain other embodiments, the cells are engineered, *ex vivo* or *in vivo*, using an adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, N., Curr. Topics in